University of Alberta

Characterization of *Fusarium* pathogens of lupin in central Alberta, Canada

©

by

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Abstract

Narrow-leafed lupin (*Lupinus angustifolius* L.) is a new crop being evaluated for production in Alberta, Canada. Fusarium root rot has been implicated as the main disease of lupins in Alberta. Examination of *Fusarium* recovered from diseased lupins showed that *F. avenaceum* was the most common and pathogenic species. A wide range in the pathogenicity of *F. avenaceum* toward lupins was found. *Fusarium avenaceum* was found to be highly diverse genetically with two equally common and closely related groups existing throughout the province. There was no relation between genotype and the pathogenicity or the origin of the isolate. Both mating types occurred within the province. Multilocus analyses for linkage disequilibrium showed that recombination has occurred within *F. avenaceum* and that the species maybe reproducing sexually. *Fusarium avenaceum* was found to be capable of causing disease in lupins alone and did not form a disease complex. The species also had an extremely broad host range, capable of causing disease on many crop species other than lupin.

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Table of Contents

1. Literature review 1

- 1.1 The *Lupinus* genus 1
  - 1.1.1 Lupins as crop plants 1
    - 1.1.1.1 Origins of lupins as crops 2
    - 1.1.1.2 Recent history of lupins as crop plants 3
- 1.2 The *Fusarium* genus 6
- 1.3 Fusarium diseases of lupin 8
  - 1.3.1 Fusarium wilt 8
    - 1.3.1.1 Races of *F. oxysporum* f. sp. *lupini* 8
    - 1.3.1.2 Host specificity of *F. oxysporum* f. sp. *lupini* 10
    - 1.3.1.3 Distribution of *F. oxysporum* f. sp. *lupini* 10
  - 1.3.2 Root and hypocotyl rots and damping-off of lupin caused by *Fusarium* spp. 11
    - 1.3.2.1 Regional differences in *Fusarium* root diseases of lupins 11
- 1.4 *Fusarium avenaceum* 12
  - 1.4.1 Ecology and pathology 12
  - 1.4.2 Taxonomy and phylogeny 13
- 1.5 Research objectives 14
- 1.6 Literature cited 16

2. Evaluation of *Fusarium* spp. causing diseases of lupin in Alberta 31

- 2.1 Introduction 31
- 2.2 Materials and methods 32
  - 2.2.1 Isolation and identification of possible fungal pathogens 32
  - 2.2.2 *Fusarium* isolates and identification 32
    - 2.2.2.1 *Fusarium* isolates 32
    - 2.2.2.2 Identification of *Fusarium* isolates 33
  - 2.2.3 Screening for pathogenic *Fusarium* isolates 33
  - 2.2.4 Fusarium wilt pathogenicity assays 35
    - 2.2.4.1 Root dip inoculations with cultivars Arabella and Rose 35
    - 2.2.4.2 Continuous dip inoculations 35
    - 2.2.4.3 Long term pathogenicity test with lupin variety FBRF 36
  - 2.2.5 Statistical analysis 37
    - 2.2.5.1 Pathogenicity screenings 37
    - 2.2.5.2 Root dip inoculations with Arabella and Rose 37
    - 2.2.5.3 Continuous dip inoculations 38
    - 2.2.5.4 Long term pathogenicity test with FBRF 38
- 2.3 Results 38
  - 2.3.1 Isolation and identification of possible fungal pathogens 38
  - 2.3.2 Screening for pathogenic *Fusarium* isolates 39
  - 2.3.3 Fusarium wilt pathogenicity assays 40
    - 2.3.3.1 Root dip inoculations with Arabella and Rose 40
    - 2.3.3.2 Continuous dip inoculations 40
    - 2.3.3.3 Long term pathogenicity test with FBRF 40
- 2.4 Discussion 40
- 2.5 Literature cited 54

3. Genetic diversity and possibility of recombination in *Fusarium avenaceum* isolated from lupins in central Alberta 59
- 3.1 Introduction 59
- 3.2 Materials and methods 61
  - 3.2.1 Isolates 61
  - 3.2.2 DNA extraction 61
  - 3.2.3 RAPD and MP-PCR analysis 61
  - 3.2.4 Fusarium avenaceum mating-type idiomorph analysis 63
  - 3.2.5 Relationship between genetic diversity, mating type, and pathogenicity 63
- 3.3 Results 63
  - 3.3.1 RAPD and MP-PCR analysis 63
  - 3.3.2 *Fusarium avenaceum* mating-type idiomorph analysis 64
  - 3.3.3 Analysis of recombination within *F. avenaceum* populations 64
  - 3.3.4 Relation of genetic diversity and mating type to pathogenicity. 65
- 3.4 Discussion 65
- 3.5 Literature cited 73

4. Characterization of the pathogenicity of *Fusarium avenaceum* 80
- 4.1 Introduction 80
- 4.2 Materials and methods 80
  - 4.2.1 Influence of *F. avenaceum* inoculum density on lupin root rot and nodulation 80
  - 4.2.2 Yield loss model 82
  - 4.2.3 Coinoculation of lupin with *F. avenaceum* and other *Fusarium* spp. 82
    - 4.2.3.1 Greenhouse assay 82
    - 4.2.3.2 Plate confrontation assay 84
  - 4.2.4 Pathogenicity of *F. avenaceum* isolates on different lupin genotypes 84
  - 4.2.5 *Fusarium avenaceum* host range 85
  - 4.2.6 Statistical analysis 85
    - 4.2.6.1 Inoculum density and nodulation 85
    - 4.2.6.2 Yield loss model 86
    - 4.2.6.3 Coinoculation of lupin with *F. avenaceum* and other *Fusarium* spp. 86
      - 4.2.6.3.1 Greenhouse assay 86
      - 4.2.6.3.2 Plate confrontation assay 86
    - 4.2.6.4 Pathogenicity of *F. avenaceum* isolates on different lupin genotypes 86
    - 4.2.6.5 *Fusarium avenaceum* host range 87
- 4.3 Results 87
  - 4.3.1 Influence of *F. avenaceum* inoculum density on lupin root rot and nodulation 87
  - 4.3.2 Yield loss model 88
  - 4.3.3 Coinoculation of lupin with *F. avenaceum* and other *Fusarium* spp. 88
    - 4.3.3.1 Greenhouse assay 88
    - 4.3.3.2 Plate confrontation assay 89
  - 4.3.4 Pathogenicity of *F. avenaceum* isolates on different lupin genotypes 89
  - 4.3.5 *Fusarium avenaceum* host range 90
- 4.4 Discussion 91
  - 4.4.1 Influence of *F. avenaceum* inoculum density on lupin root rot and nodulation 91
  - 4.4.2 Yield loss model 91

4.4.3 Coinoculation of lupin with *F. avenaceum* and other *Fusarium* spp. 91
4.4.4 Pathogenicity of *F. avenaceum* isolates on different lupin genotypes 93
4.4.5 *Fusarium avenaceum* host range 94
4.5. Literature cited 115
5. General discussion 121
5.1 Literature cited 124

List of Tables

Pages

Table 2-1. Frequency of fungi isolated from taproot samples collected from experimental plantings of lupin in central Alberta in 2006……………………………………………47

Table 2-2. *Fusarium* spp. isolated from lupin roots collected from experimental plantings of lupin in central Alberta in 2005 and 2006…………………………………………………48

Table 2-3. Test statistics for the effects of *Fusarium* isolate and cultivar on emergence, root rot severity, and shoot weight of lupin………………………………………………49

Table 2-4. Pathogenicity of *Fusarium* isolates towards lupin seedlings……………………50

Table 2-5. Wilt severity for different lupin varieties inoculated with *F. oxysporum* f. sp. *lupini* race 3 isolate CBS 280.8 / IMI 141142, in a continuous dip assay…………………53

Table 2-6. Wilt severity of lupin variety FBRF inoculated with different *F. oxysporum* isolates……………………………………………………………………………53

Table 3-1. Primers used for the characterization of monoconidial *Fusarium* isolates collected from lupin in central Alberta and number of DNA bands analyzed…………………………70

Table 3-2. Primers used for the molecular determination of the mating type of *F. avenaceum* isolates collected from lupin in central Alberta……………………………………………70

Table 3-3. Tests for linkage equilibrium amongst *F. avenaceum* isolated collected from lupin in central Alberta…………………………………………………………………72

Table 4-1. Isolates used for the comparison of the pathogenicity of *F. avenaceum* on different lupin genotypes…………………………………………………………………97

Table 4-2. Correlation of the number of nodules per root of lupin cv. Arabella or Rose with *F. avenaceum* inoculum density, root rot, shoot weight, and root weight for isolates 213 and 371........101

Table 4-3. Effect of artificial *F. avenaceum* seed contamination on lupins in 2006 at Lacombe, AB, Canada and in 2007 at Edmonton, AB, Canada........101

Table 4-4. Inhibition of *F. avenaceum* by other *Fusarium* spp. isolated from lupins in dual culture on potato-dextrose agar (PDA) medium........106

Table 4-5. PROC MIXED test statistics for the effect of lupin genotype and *Fusarium* isolate on dry shoot weight, root rot severity, disease incidence, and emergence in the greenhouse........106

Table 4-6. Shoot weights, root rot, disease incidence, and emergence of five lupin genotypes inoculated with *F. avenaceum*........111

Table 4-7. Effect of soil inoculation with *Fusarium avenaceum* on emergence, root rot severity, root length, plant height, fresh weight, and dry weight of eighteen crop species........112

List of Figures

Pages

Figure 2-1. Locations of lupin plots where *Fusarium* isolates were collected……………46

Figure 2-2. Recovery *Fusarium* spp. from roots of lupins in 2006…………………………47

Figure 2-3. Frequency of common *Fusarium* spp. grouped according to their effect on lupin emergence, root rot, and shoot dry mass……………………………………………51

Figure 3-1. UPGMA dendrogram of similarities between *Fusarium* spp. recovered from lupins in central Alberta……………………………………………………………71

Figure 4-1. Diagram of the agar plate confrontation assay…………………………………96

Figure 4-2. Relationship between inoculum density of *Fusarium avenaceum* and lupin A) emergence, B) root rot, C) nodulation, D) shoot dry weight, and E) root dry weight…100

Figure 4-3. The effect of *Fusarium* isolates alone and in combination with *F. avenaceum* (2×10^4 CFU g^{-1}) on *L. angustifolius* cv. Arabella…………………………………103

Figure 4-4. The effect of *Fusarium* isolates alone and in combination with *F. avenaceum* (5×10^3 CFU g^{-1}) on *L. angustifolius* cv. Arabella…………………………………105

Figure 4-5. Reaction of five lupin genotypes inoculation with *Fusarium*…………………108

Figure 4-6. Reaction of lupins to twelve *F. avenaceum* isolates, two *F. acuminatum* isolates, and a control……………………………………………………………………110

1. Literature review

1.1 The *Lupinus* genus

Lupins are members of the family Fabaceae, the subfamily Papilionoideae, the tribe Genisteae, and the genus *Lupinus* L. Two to five hundred lupin species are recognized (Dunn and Gillett 1966). Most are annual herbaceous species, but there are some herbaceous or shrubby perennial species (Gladstones 1998). The vast majority of the species occur in the New World in alpine, temperate and subtropical areas from Alaska to southern Argentina and Chile. Only twelve species are native to the Old World and originate around the Mediterranean and East Africa (Gladstones 1998). All are herbaceous annuals. Lupin species typically grow on well drained acidic or neutral soils with a course texture. Gladstones (1970) describes naturalized lupins as typically growing in poor, sandy soils and rarely on alkaline or calcareous soils.

Lupins, like many other leguminous plants, are capable of forming a nitrogen-fixing symbiosis with facultative microsymbiont rhizobia bacteria, which fix atmospheric nitrogen within root nodules. Lupins are nodulated by *Bradyrhizobium* strains known as *Bradyrhizobium* sp. *(Lupinus)*. The *Bradyrhizobium* capable of nodulating lupins may belong to multiple species (Jarabo-Lorenzo 2003). *Bradyrhizobium* sp. (*Lupinus*) is acid- and aluminium-tolerant and possibly an acidophile (Howieson et al. 1998). The relationship between lupins and their symbionts is relatively unique among legume crops. Few other temperate crops have *Bradyrhizobium* as a symbiont and the initiation of the symbiosis and mode of entry of the bacterium into the roots is also distinctive (Howieson et al. 1998). The resulting nodules are indeterminate, but due to differing morphology from other indeterminate nodules, they have been described as 'lupinoid' (Corby 1981).

1.1.1 Lupins as crop plants

The use of lupins as a crop species has been limited mainly to three Old World species, *Lupinus albus* L. (white lupin), *L. luteus* L. (yellow lupin), and *L. angustifolius* L. (blue or narrow-leafed lupin), and one New World species *L. mutabilis* Sweet (Andean lupin). *Lupinus luteus* and *L. angustifolius* have been shown to be the most closely related of the four species, belonging to the same monophyletic group, based on the internal transcribed space (ITS) sequences of their nuclear ribosomal DNA (Ainouche and Bayer 1999).

As crop plants lupins provide both direct and indirect agronomic benefits. Lupin seeds have been used as a fodder and a forage crop for livestock, and due to the high protein content have found a market as an animal feed supplement (Cox 1998; Helgadóttir et al. 2004; von Baer 2006). Lupins also provide a number of benefits to subsequent crops when used in rotation. The primary benefit comes from the high potential of lupins for nitrogen fixation and dissolving phosphorus and other minerals in the soil (Hondelmann 1984; Palmason et al. 1992). Therefore, lupin crops have often been used as green manure to provide for better yields of subsequent crops (Evans et al. 1989; Unkovich et al. 1994; Vellasamy et al. 2000). Rotating crops with lupins can also have a cleansing effect, reducing the severity of diseases affecting cereal crops (Wilson and Hamblin 1990). The deep roots of lupins also aid in subsoil loosening, which may increase water availability for subsequent crops (Henderson 1989).

1.1.1.1 Origins of lupins as crops

The cultivation of lupin as a crop is known to date back to antiquity, with cultivation of *L. albus* in Egypt around 2000 B.C. (Gladstones 1970). By the classical Greek and Roman periods, white lupin cultivation was occurring in multiple areas in the Mediterranean (Hondelmann 1984). The crops were used as a green manure, in rotation with wheat to improve wheat yields, and on marginal and poorly cultivated lands. Archaeological records show South-American *L. mutabilis* cultivation dates back to the 7th century B.C. (Hondelmann 1984). The early lupin varieties that were cultivated in both the Old and New World were undomesticated, having hard seed coats, shattering pods that prevented efficient harvesting, and contained high alkaloid levels ('bitter seeds') that prevented their consumption without prior debittering (Hondelmann 1984). Debittering required extracting the alkaloids from the seeds in water, alcohol, or other solvents.

1.1.1.2 Recent history of lupins as crop plants

The expansion of lupin production into northern Europe is attributed to Fredrick the Great of Prussia, who personally ordered the importation of *L. albus* into Prussia from Italy (Hondelmann 1984). The intent was to improve the poor sandy soils of northern Prussia. The selected cultivars grew poorly in the Prussian climate, and production declined dramatically after Fredrick's death in 1786. A farmer introduced yellow lupin into Prussia in 1841. By 1860, yellow lupin production had spread across the acidic and sandy soils of Prussia. *Lupinus angustifolius* was introduced into Prussia, northern Europe, and England around this time, probably from the Iberian peninsula (Oldershaw 1920; Gladstones 1998). Lupin production, dominated by yellow lupin, stabilized at ~400 000 ha in northeast Germany, mostly for forage and green manure (Hondelmann 2000). The better frost tolerance of *L. angustifolius* encouraged its increased cultivation in northern latitudes. Production of *L. angustifolius* and *L. luteus* spread across eastern Europe into northern Ukraine and Russia (Gladstones 1998). Lupin production declined in the early 1900s, due in part to the increased use of nitrogen fertilizers, the importation of feed protein, and a decline in the wool industry that resulted in a reduced demand for forage (Hondelmann 2000). Interest in lupin increased with the outbreak of World War I and the resultant blockade of Germany, as there was a shortage of protein for human consumption. Lupin production increased and attempts to breed an alkaloid-free (sweet) lupin intensified (Hondelmann 2000). In the late in 1920s, the first sweet yellow and narrow-leafed lupin cultivars were selected in Germany (Hondelmann 2000). Natural mutants of yellow lupin with permeable seed coats and nonshattering pods were found in Germany during the 1920s and 1930s (Cowling et al. 1998). Narrow-leafed lupins with permeable seed coats were discovered in lines of unknown origin in the 1930s (Cowling et al. 1998).

From Germany, narrow-leafed lupin was exported to other countries including the U.S.A., South Africa, South America and New Zealand (Hondelmann 1984; Gladstones 1998). All three old world species were exported to southeastern states in the U.S.A (Prince and Chambliss 2004; Noffsinger and van Santen 2005). *L. angustifolius* became an important winter green manure crop in Florida, South Carolina, and other southeastern states from the 1930s until the early 1950s, when lupin plantings peaked at over one million hectares (Armstrong and Armstrong 1964; Putnam 1993; Prince and Chambliss 2004). Severe winter frosts and reduced costs of nitrogen fertilizers caused lupin to cease to be an important crop in the U.S.A. (Putnam 1993; Prince and Chambliss 2004). In Europe, narrow-leafed lupin production and research shifted to Poland, East Germany, and other eastern European countries after World War II (Hondelmann 1984; Brummund and Bornhof 2000). Pod shattering, low germination and disease all reduced yields and hindered narrow-leafed lupin production, and East Germany discontinued sweet narrow-leafed lupin breeding in the 1960s leaving Polish, Ukrainian, and Russian institutes as the main centres of lupin research in Europe (Brummund and Bornhof 2000).

Lupinus angustifolius did not become fully domesticated until the 1960's in Australia (Cowling et al. 1998). A lupin breeding program was started in Australia in the 1950s. In 1960, mutant bitter plants that had non-shattering pods were discovered (Gladstones 1967). In 1967 the first sweet varieties were introduced (Cowling et al. 1998). This development led to the increased production of lupins in rotation with wheat on the poor sandy soils of Western Australia.

European lupin production declined severely during the 1970s, mainly due to high losses caused by Fusarium wilt and the replacement of lupins with cereal crops. The recent history of *L. angustifolius* production has been dominated by Australia. In the mid 1980's, Australian lupin seed production accounted for only 26% of the worlds total lupin seed harvests of 518 000 tonnes (Pate et al. 1985). By the early 1990s, lupin seed production more than doubled to 1 million tonnes with about 78% grown in Australia (Cox 1998). Unlike European lupin production, Australian lupin cultivation is dominated almost exclusively by *L. angustifolius*. Western Australia has emerged as the main lupin producing state in Australia with 500 000 to 1 000 000 tonnes of *L. angustifolius* seed produced each year, more than any other country in the world (French and Buirchell 2005). Continued breeding efforts in Australia have resulted in cultivars with yields two to three times higher then the original sweet lupin cultivars released in 1976 (French and Buirchell 2005).

Outside of Australia, there has been a recent increase in interest in *L. angustifolius* and other lupin species. The breeding of new cultivars better adapted to northern climates has led to attempts to produce lupins in areas where they were never grown or ceased to be grown as a crop (Joernsgaard et al. 2004; Kurlovich et al. 2004). Breeding of new disease resistant cultivars has also allowed lupin to be produced in areas where it was not previously viable, because of high disease pressure (Kuptsov 2000; Kutpsov et al. 2006). The spread of anthracnose of lupin into Europe has also encouraged the cultivation of narrow-leafed lupin instead of white or yellow lupin due to better disease resistance in narrow-leafed lupin (Cowling et al. 2000). A nearly complete switch to narrow-leafed lupin from white and yellow lupin occurred in Germany due to disease outbreaks (Frick et al. 2004), and white and yellow lupin cultivars have been abandoned in favour of *L. angustifolius* in central and eastern Europe (Cowling et al. 2000; Frencel 2004). The natural ability of narrow-leafed lupin to act as a green manure crop has led to its inclusion in numerous organic or sustainable agriculture programs (Helgadóttir et al. 2004; Danish Research Centre for Organic Food and Farming (DARCOF) 2005b; Boström 2006). Recent European regulations excluding the use of animal protein in feed has also led to an increased interest in narrow-leafed lupin as a protein crop (Frick et al. 2004).

In the Americas, there has also been a surge in interest in lupin as an alternative crop, mainly due to its use in sustainable agriculture and as an alternative protein crop. In Chile narrow-leafed lupin production has reached ~7 000 ha per year, with most of the cultivars grown originating from Australia (von Baer 2006). Despite the decline of American lupin production in the 1950s and discontinuation of the Georgian narrow-leafed lupin breeding program in the 1980s, lupin research continues. In the U.S.A. lupin research has been conducted recently in Alabama, Michigan, North Dakota, Washington, Wisconsin, Virginia, and Oregon (Copeland 1998; Payne et al. 2004; Noffsinger and van Santen 2005). Most of the trials focused on white lupin, but narrow-leafed lupin was tested in Oregon and Virginia.

The first attempts to grow *L. angustifolius* as a crop in Canada took place in Manitoba in the 1970s (Oomah and Bushuk 1984). More recent attempts have occurred in Alberta and Nova Scotia (Blade et al. 2004; Organic Agriculture Centre of Canada (OACC) 2006). Albertan lupin research has focused on new European *L. angustifolius* genotypes (Blade et al. 2004). The pulse industry has attempted to increase the representation of legume crops in crop rotation and narrow-leafed lupin could fulfil this role (Blade et al. 2004). It is estimated that Alberta alone could support 141 000 ha of narrow-leafed lupins and the crops tolerance for acidic and poor soils could allow the expansion of the pulse industry into areas that cannot currently support pulse crops (Blade et al. 2004; Lopetinsky et al. 2006). A potential market for lupin grain exists in the fish feed market and it could help to reduce soybean imports (Lopetinsky et al. 2006).

1.2 The *Fusarium* genus

Fusarium is an anamorphic genus of filamentous ascomycetes. It contains three teleomorph genera, *Gibberella*, *Haematonectria*, and *Albonectria* (Leslie and Summerell 2006). *Gibberella* constitutes the majority of *Fusarium* species. Collectively, *Fusarium* has a worldwide distribution and is associated with many diseases of numerous plant species (Booth 1971), some diseases of animals and humans (Nelson et al. 1994; Mehl and Epstein 2007), and the production of mycotoxins (Tóth et al. 2004; Uhlig et al. 2007).

Fusarium species are delimitated based on the morphological, biological, and phylogenetic species concepts or a combination of multiple concepts (Leslie et al. 2001). *Fusarium* was first described as a genus by Link in 1809, with the main characteristic being canoe-shaped macroconidia. Within one hundred years, there were over one thousand species, varieties, and forms named on the basis of superficial observations (Toussoun and Nelson 1975).

The modern separation of *Fusarium* into species based on morphology began with the work of Wollenweber and Reinking. The researchers published their taxonomy of the Fusarium in *Die Fusarien* (Wollenweber and Reinking 1935). They reduced the approximately 1 000 named *Fusarium* species into 16 sections with 65 species occurring within the sections. There were also 55 varieties and 22 forms recognized. Before this work, some species such as *F. avenaceum* (Fries) Saccardo had been assigned 77 different synonyms for species names (Wollenweber and Reinking 1935). Wollenweber and Reinking (1935) used a variety of morphological characters including the presence or absence and characteristics of conidia (microconidia and macroconidia) and chlamydospores to determine sections and a variety of other characters to separate species, varieties and forms. The sections created by Wollenweber and Reinking are often not monophyletic, but many remain in use currently (Leslie et al. 2001). Many alternative morphology-based taxonomic systems for *Fusarium* followed, with different numbers of species and sections. Snyder and Hansen (1940, 1941, 1945) reduced the genus to nine species. This classification system was popular in America and elsewhere during much of the 20th century. The severe reduction in *Fusarium* species has been blamed for a loss of information, which has rendered some of the data generated during this time difficult to interpret or evaluate (Leslie and Summerell 2006). The largest loss of information occurred when Snyder and Hansen combined four of Wollenweber and Reinking's sections (*Arthrosporiella*, *Discolor*, *Gibbosum*, and *Roseum*) and the twenty-two species therein into the single species, *F. roseum* (Link.). The next major change in *Fusarium* taxonomy came as a result of the work of Booth (1971) who based his system on the work of Wollenweber and Reinking and other researchers (Leslie et al. 2001). Booth's system recognized twelve sections and forty-four species. *Fusarium roseum* of Snyder and Hansen's system was subdivided into seventeen separate species. The next two major changes to Fusarium taxonomy were those of Gerlach and Nirenberg (1982) in Germany and Nelson et al. (1983) in the U.S.A. Both groups of authors used sections developed by Wollenweber and Reinking, but Gerlach and Nirenberg recognized 78 species whereas Nelson et al. recognized 30 species. The two systems have become the dominant systems for identifying *Fusarium* spp. and describing new taxa (Leslie et al. 2001; Leslie and Summerell 2006).

The biological species concept has had limited application within the *Fusarium* genus, due to many species not reproducing sexually. Some species, such as members of the section *Liseola* have been distinguished based on reproduction. The recognition of *Fusarium* species based on the phylogenetic species concept has been increasing with the adoption of molecular methods, and as a result the number of recognized *Fusarium* species has increased. Multilocus DNA seqencing has shown that some well recognized morphological species are composed of multiple phylogenetic species. In the case of *F. graminearum* Schwabe, eleven different phylogenetic species have been recognized within the species (O'Donnell et al. 2004; Starkey et al. 2007). The different species concepts can conflict with each other. Isolates of *F. graminearum* that have been placed in different phylogenetic species are cross-fertile *in vitro*, which would make them the same biological species (Leslie et al. 2001; Starkey et al. 2007).

1.3 *Fusarium* diseases of lupin

Most *Fusarium* diseases of lupin can be divided into two types: the vascular wilts and root diseases including root rot, hypocotyl rot, and damping off. Pod rot of lupin, caused by *Fusarium*, has also been found (Richter 1941).

1.3.1 Fusarium wilt

Fusarium wilt is caused by members of the species *Fusarium oxysporum* Schlechtendahl emend. Snyder & Hansen. *Fusarium oxysporum* is an anamorphic species based on a series of morphological characters (Nelson et al. 1983), but is probably a polyphyletic species group (Leslie et al. 2001). It is the most common and widely distributed *Fusarium* species (Pietro et al. 2003; Leslie and Summerell 2006). From a phytopathological perspective, the species is best known for its ability to cause vascular wilt diseases. The fungus penetrates plant roots, proceeds intercellularly through the root cortex until it reaches the xylem vessels, then spreads through the host via the xylem (Pietro et al. 2003). The subsequent wilting symptoms and mortality that often occur result from severe water stress, believed to be caused by a combination of pathogen growth and host defence responses within and surrounding the vascular tissues (Beckman 1987; Pietro et al. 2003). *Fusarium oxysporum* has been subdivided into *formae speciales* based on host specificity. Presently, over 120 *formae speciales* have been recognized (Pietro et al. 2003). *Forme speciales* are further subdivided into races, which have been defined inconsistently, but usually correspond to cultivar specificity within a species (Kistler 1997).

1.3.1.1 Races of *F. oxysporum* f. sp. *lupini*

The first report of wilt of lupin caused by *Fusarium* occurred in 1906 in Germany (Armstrong and Armstrong 1964). It was noticed at the time that although *L. angustifolius* plants were suffering from wilt, *L. luteus* plants in the same area were not affected. The characterization of the races and host range of *F. oxysporum* causing wilt of lupin would not occur until much latter. Snyder and Hansen (1940) first applied the term *lupini* to describe strains causing wilt in lupin, excluding strains causing root rot of lupin.

The recognition of the races within *F. oxysporum* f. sp. *lupini* first occurred in 1941. Richter (1941), in Germany, separated the pathogen into three races based on the pathogenicity of isolates on four lupin species: *L. albus*, *L. luteus*, *L. angustifolius*, and *L. mutabilis*. The first race was pathogenic to *L. luteus*, the second to *L. luteus* and *L. albus*, and the third race was pathogenic to all species except *L. luteus*. The cultivars used were not described. Lamberts (1955) in the Netherlands then defined three physiological races based on the reaction of different *L. luteus* cultivars and genotypes. The author did not state if his three races were related to the races of Richter. Armstrong and Armstrong (1964), in America, then re-examined the races of Richter using isolates imported from Europe using a bitter *L. angustifolius* cultivar, three *L. luteus* cultivars, and one *L. albus* cultivar. Race one and two were both found to be pathogenic on white and yellow lupin and race three was highly pathogenic to *L. angustifolius*, *L. albus*, and a weaker pathogen of one *L. luteus* cultivar. Despite no difference in host specificity between races 1 and 2, the authors maintained the three race classification system in keeping with Richter's results. Salleh and Owen (1983) retested the pathogenicity of the three races on white lupin cultivars; race three was the most virulent. A standard set of differential cultivars has never emerged despite one being suggested by Salleh and Owen (1983). The actual number of races present in Europe is probably greater than three. *L. angustifolius* lines have been found to display varying patterns of resistance and susceptibility depending on the area in which they are planted (Kurlovich et al. 1995). This could suggest differences in pathogen populations or an interaction between host, pathogen and environmental conditions.

F. oxysporum f. sp. *lupini* race 3, the only race shown to be pathogenic towards *L. angustifolius*, maybe the most genetically distinct of the three races. Vegetative compatibility group (VCG) testing of central European isolates has shown race 1 and 2 isolates to be in the same VCG as the majority of *F. oxysporum* f. sp. *lupini* isolates, whereas race 3 was self-incompatible (Rataj-Guranowska 1992). Rataj-Guranowska (1992) also found that their race 3 isolate and other self-incompatible isolates were the most aggressive pathogens. Serological testing has also shown races 2 and 3 to be distinct (Rataj-Guranowska 1988a,b). Isolates of *Fusarium redolens* Wollenweber have also been shown to be vegetatively compatible with isolates of races 1 and 2 of *F. oxysporum* f. sp. *lupini* and to cause wilt in *L. leutus* (Rataj-Guranowska et al. 1991).

1.3.1.2 Host specificity of *F. oxysporum* f. sp. *lupini*

Fusarium oxysporum f. sp. *lupini* has been shown to be very specific to *Lupinus* species. Armstrong and Armstrong (1964) tested *F. oxysporum* f. sp. *lupini* on 45 plant varieties in 36 species and found none were susceptible to the disease. They also tested 42 different wilt Fusaria on lupins and found that only alfalfa, bean, cassia, cotton races 1 and 2, and cowpea races 1 and 3 could cause wilt in certain *L. luteus* and *L. albus* cultivars, although disease development was slower and less severe than that caused by *F. oxysporum* f. sp. *lupini*. *Lupinus angustifolius* was unaffected. The effectiveness of crop rotation to control the disease also supports the fact that the disease is specific to lupin (Lamberts 1955). The fact that *L. angustifolius* is only susceptible to race 3 of *F. oxysporum* f. sp. *lupini*, which has been shown to be the race with the widest host range and greatest virulence, may indicate that *L. angustifolius* has a higher resistance to wilt.

1.3.1.3 Distribution of *F. oxysporum* f. sp. *lupini*

After its initial discovery in Germany, Fusarium wilt of lupin became an epidemic problem for lupin production in central and eastern Europe for all three cultivated lupin species. By the 1970s, lupin production was seriously affected (Pate et al. 1985). Fusarium wilt of *L. albus* has been reported to be common in areas of northern Africa and South Africa (Malan and Knox-Davies 1990; Christiansen et al. 1999). Wilt has not been identified as a problem of *L. angustifolius* in the Americas. Despite the fact that Armstrong and Armstrong (1964) showed that some cultivars of yellow and white lupin were susceptible to Fusarium wilts of other crops in America, no occurrences of wilt were ever reported. No wilt has ever been reported in Australia, despite widespread lupin cultivation and the fact that the cultivars grown have been tested in Europe and found to be susceptible (Department of Agriculture, Government of Western Australia 2004).

1.3.2 Root and hypocotyl rots and damping-off of lupin caused by *Fusarium* spp.

The main *Fusarium* species associated with root rots of lupins are *F. oxysporum* and *F. avenaceum* (Golubev and Kurlovich, 2002). A large number of other *Fusarium* species have also been shown to be able to cause root rot or seedling blight of *L. angustifolius*, including *F. culmorum* (W.G. Smith) Saccardo, *F. poae* (Peck) Wollenweber, *F. equiseti* (Corda) Saccardo, *F. solani* (Martius) Appel & Wollenweber emend. Snyder & Hansen, *F. semitectum* Berkeley & Ravenel, *F. tricinctum* (Corda) Saccardo, and *F. moliliforme sensu lato* (Wollenweber and Reinking 1935; Weimer 1944; Nowicki 1995). The species have been shown to be pathogenic in experiments, but in fields they are often isolated in combinations from plants. *Fusarium redolens* has also been implicated as a root pathogen of lupin, but its morphological resemblance to *F. oxysporum* can result in it being reported as *F. oxysporum* (Joernsgaard et al. 2004).

1.3.2.1 Regional differences in *Fusarium* root diseases of lupins

Reports of the pathogenicity of *Fusarium* species causing root rots vary between studies and areas. Within Europe, *F. avenaceum, F. oxysporum,* and *F. solani* are commonly reported as root pathogens of lupins. *Fusarium avenaceum* has been found to cause root rot and seedling blight of lupin species in the United Kingdom (Bateman 1997) in the west, through central Europe (Wollenweber and Reinking 1935), and into Russia in the east (Debelyi et al. 1977). *Fusarium solani* has been reported to cause root rot in the U.K. (Bateman 1997), and in areas of Denmark and Belarus; *F. oxysporum* and *F. solani* have been reported to be significantly more aggressive than *F. avenaceum* (DARCOF 2005).

When the southeastern states of the U.S.A. were major lupin producing areas, most root and hypocotyl rot-causing *Fusarium* species were *F. oxysporum*, *F. solani*, and *F. moniliforme sensu lato* (Weimer 1944). In more northern areas of the U.S.A., *F. oxysporum* and *F. avenaceum* have been implicated as the main *Fusarium* pathogens, with *F. solani*, *F. moniliforme sensu lato*, and *F. acuminatum* being less important (Kalis 1990). In Australia, currently the main *L. angustifolius*-producing region, *Fusarium* diseases have not been reported to be major problems despite the species being associated with lupins (Sweetingham 1989).

1.4 *Fusarium avenaceum*

Fusarium avenaceum is an anamorphic heterothallic ascomycete found in soil in temperate regions (Leslie and Summerell 2006). Its teleomorph, *Gibberella avenacea* (Cook), has only been reported twice, once in Washington, U.S.A. on wheat (Cook 1967) and once in the U.K on *Pteridium aquilinum* (Booth and Spooner 1984).

Colonies of *F. avenaceum* exhibit a wide range of variation in colour and appearance (Booth 1971) with mutant degenerate forms being relatively common (Leslie and Summerell 2006). Microscopically, *F. avenaceum* is distinguished by its long and slender macroconidia, its rare production of microconidia, and its lack of chlamydospores.

1.4.1 Ecology and pathology

F. avenaceum is a common soil fungus. It is found in both agricultural and non-agricultural soils (Kommedahl et al. 1988; Hestbjerg et. al 1999) and can be one of the main *Fusarium* species in agricultural soil. *Fusarium avenaceum* is considered both a saprophyte and a pathogen. As a saprophyte, it can survive on crop debris within the soil and on the soil surface (Hudson 1968; Hestbjerg et al. 1999). It also occurs as a common saprophyte accompanying important root pathogens (Summerell et al. 2003). Despite its classification as a saprophyte, it has also been found growing endophytically within the roots of nondiseased plants (Jenkinson and Parry 1994b). The species host range is extremely large, and it has been isolated from over 160 different host genera (Booth 1971). As a pathogen it causes a variety of diseases and infects a wide range of plant species, from potatoes to pine trees (Satyaprasad et al. 1997; Asiegbu et al. 1999). Individual isolates can exhibit broad host ranges, infecting plants from different families (Schneider 1958; Satyaprasad et al. 1997). The most common diseases caused by *F. avenaceum* are blights, root rots, and ear rot of cereals (Desjardins 2003; Leslie and Summerell 2006), but it also causes dry rot of potato, storage diseases of vegetables, and stem rots. Certain legumes, such as lentils and subterranean clover, appear to be the most susceptible crops to root rot caused by *F. avenaceum* (Leslie and Summerell 2006). On cereals, *F. avenaceum* is involved in Fusarium Head Blight (FHB) and is commonly associated with *F. graminearum* Schwabe, *F. culmorum*, and *F. pseudograminearum* Aoki & O'Donnell, (Parry et al. 1995; Yli-Mattila et al. 2004). Although it is typically a minor member of the FHB complex, it has been found to be dominant in eastern Europe and Scandinavia (Xu et al. 2005; Uhlig et al. 2007)

Although considered a soil-borne fungus there is evidence that *F. avenaceum* is capable of spreading by several means. Splash-dispersion maybe an effective dispersion method over short distances (Jenkinson and Parry 1994a). Over longer distances, a variety of mechanisms maybe employed. It has been shown to be a seed borne pathogen on some crop species (Kollmorgen 1974; McGee and Kellock 1974). *Fusarium avenaceum* has been isolated from the exterior of a variety of insects (Gordon 1959; Windels et al. 1976) and more recently, it has been shown that some insect species are attracted to *F. avenaceum* sporodochia and will vector macroconidia, internally and/or externally, to other plants resulting in infection (El-Hamalawi and Stanghellini 2005). It may also be airborne, as *F. avenaceum* propagules have been recovered in spore traps sampling the air above fields, and it can be the dominant *Fusarium* species present (Martin 1988).

1.4.2 Taxonomy and phylogeny

The taxonomy of *Fusrarium avenaceum* has been fairly consistent since Wollenweber and Rinking's initial description of the species as a member of the section *Roseum*. Snyder and Hansen (1945) did combine *F. avenaceum* and many other species into *F. roseum*, but most other taxonomies have kept *F. avenaceum* as a separate species. Gordon (1952), in Canada, recognized *F. avenaceum* as a species, within the section *Roseum*, in his taxonomy. Booth (1971) recognized *F. avenaceum* as a species, but within the section *Arthrosporella* as his classification had no section *Roseum*. Gerlach and Nirenberg (1982) and Nelson et al. (1983) both placed *F. avenaceum* as a species within the section *Roseum*. Nelson et al. (1983) placed *F. arthrosporioides* within *F. avenaceum* on a provisional basis, until it could be determined if there was a clear demarcation between the species. *F. arthrosporioides*, a morphologically similar species, was considered a separate species from *F. avenaceum* by Wollenweber and Rinking (1935) and Gerlach and Nirenberg (1982) due to the lack of bright orange sporodochia and the presence of pyriform conidia. The authors did recognize the similar morphology of the two species. Some researchers treat *F. arthrosporioides* as a synonym of *F. avenaceum* (Kirstensen et al. 2005)

Fusarium avenaceum, a producer of the mycotoxin moniliformin, has been shown to be closely related to other moniliformin-producing species including *F. tricinctum*, *F. torulosum* (Berkeley & Curtis) Nirenberg, and *F. flocciferum* (Kirstensen et al. 2005). Many Fusarium species such as *F. oxysporum* and *F. solani* are species groups that actually contain multiple phylogenetic or biological species (O'Donnell et al. 1998; O'Donnell 2000). Amongst phylogeneticists there has been some debate as to whether *F. avenaceum* is a monophyletic species or polyphyletic species group. Differences in morphology, internal transcribed spacer (ITS), nuclear ribosomal intergenic spacer (IGS), mitocondrial small subunit (mtSSU) rDNA, and β-tubulin sequences and universally primed polymerase chain reaction (UP–PCR) hybridization of *F. avenaceum*, *F. arthrosporioides*, *F. anguioides*, *F. tricinctum*, *F. graminum*, and *F. acuminatum* supported the grouping of *F. anguioides*, *F. avenaceum*, and some *F. arthrosporioides* strains into the same species (Yli-Mattila et al. 2002). Isolates identified as *F. arthrosporioides* had previously been considered as isolates of *F. avenaceum* by some researchers (Nelson et al. 1983). Further phylogenetic analysis by Yli-Mattila et al. (2004) found that beta tubulin, IGS, and ITS sequences of European *F. avenaceum*, *F. arthrosporioides*, *F. anguioides*, and *F. tricinctum* did not separate the species into separate monophyletic groups, and concluded some isolates may represent their own species or recently evolved phylogenetic species that have not yet separated from each other. Analysis of a worldwide collection of *F. avenaceum* isolates, dominated by American isolates, by Nalim (2004) indicated that *F. avenaceum* is highly diverse yet monophyletic. The author apparently included *F. arthrosporioides* as a synonym of *F. avenaceum.*

1.5 Research objectives

From initial investigations of lupins in experimental plots in Alberta, it is known that root rot caused by *Fusarium* spp. is the main disease of lupins regionally (Chang et al. 2005 and 2006). The objectives of this study were to identify which *Fusarium* species were capable of causing disease in lupins, determine if Fusarium wilt occurs, and characterize the dominant *Fusarium* pathogen. *Fusarium* isolates recovered from diseased lupins in 2005 and 2006 were tested for their ability to cause root rot or wilt. Further studies were undertaken to examine the dominant pathogen, *F. avenaceum*, including: analysis of the genetic diversity within *F. avenaceum* and its relationship to aggressiveness; the possibility of sexual reproduction within populations; the effect of inoculum concentration on disease development and nodulation; the effect of seed inoculation on disease development; the combined effect of *F. avenaceum* and other *Fusarium* species on the severity of disease, and the susceptibility of other crop species to this pathogen.

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2. Evaluation of *Fusarium* spp. causing diseases of lupin in Alberta

2.1 Introduction

Recently lupin has been evaluated as a potential pulse crop in central Alberta, with the goal of expanding pulse production onto acidic soils and diversifying crop rotations (Blade et al. 2004). In 2004 and 2005, Fusarium root rot was found to be the main disease of lupins in Alberta (Chang et al. 2005, 2006b). Fusarium root rot of lupin results in reddish brown lesions, which can be large enough to girdle the main tap root or cover the entire root. The lesions can be shallow, penetrating only the cortex, or can be deeper, extending into the vascular tissue. Mortality can occur in cases of severe rot. Lupins in the United States were found to suffer from root rot caused by *F. oxysporum*, *F. solani*, *F. moniliforme sensu lato*, and *F. avenaceum* (Weimer 1944; Kalis 1990). In Europe, lupin has been shown to be adversely affected by *Fusarium* spp., particularly *F. avenaceum*, *F. oxysporum* and *F. solani*. All these species can cause seedling blights and root rot while *F. oxysporum* is also responsible for Fusarium wilt of lupin. Fusarium wilt is caused by *F. oxysporum* isolates that penetrate the roots and then cause tracheomycosis upon colonization of the vascular tissue. Death due to blockage of the vascular tissue then follows. Fusarium wilt became the most harmful soil-borne disease of lupins in eastern Europe (Kurlovich et al. 1995) and caused wide spread losses (Pate et al. 1985). Within all *Fusarium* species that are common pathogens of lupins, non-pathogenic strains are also known to occur. Currently, there is little information as to which *Fusarium* species are responsible for diseases of lupins in Alberta.

The objectives of this study were to determine which *Fusarium* spp. cause most diseases of lupins in Alberta, and to determine which *Fusarium* spp. are pathogenic to lupins and which ones are only weak pathogens or saprophytes. A series of experiments were also performed to determine if Fusarium wilt of lupin occurs in Alberta, due to the importance of the disease in other lupin growing regions.

2.2 Materials and methods

2.2.1 Isolation and identification of possible fungal pathogens

In July 2006, *L. angustifolius* cv. Arabella plants displaying disease symptoms were collected from experimental field plots at two locations in Edmonton (Crop Diversification Centre North (CDCN) and Ellerslie) and Westlock, Alberta (Fig. 2-1). Plants that exhibited wilting, stunted growth or chlorosis were pulled from the soil and placed in paper bags for transport back to the laboratory. Roots were washed under running tapwater and cut into pieces ~2 mm long per side, then surface sterilized in 0.6% sodium hypochlorite for 1 min and rinsed in sterile distilled H_2O (sdH_2O) (Hwang et al. 1994). The tissue pieces were dried in glass Petri dishes and up to ten pieces were plated on ½ strength Potato Dextrose Agar (½PDA) (BD/Difco, Sparks, MD). Stems were cut longitudinally to check for any signs of vascular discolouration. If present, tissue pieces were excised, sterilized in the same manner as root pieces and plated on ½PDA. Cultures were incubated for three to four days in the dark at room temperature. Cultures were then subcultured, if necessary, to PDA acidified with lactic acid to pH 5.0 (APDA) or PDA with streptomycin. Isolates were identified to genus based on their colony morphology, or if necessary, using microscopic observations and the key of Barnett and Hunter (1998).

2.2.2 *Fusarium* isolates and identification

2.2.2.1 *Fusarium* isolates

A collection of 130 *Fusarium* isolates from lupin was compiled from fungal isolates recovered from Arabella in 2006 at the locations described above. An additional 116 isolates, provided by Dr. K.F. Chang (Alberta Agriculture and Rural Development, Lacombe) were also included in the study. These isolates were recovered from *L. angustifolius* cv. Arabella in 2005 from plots located in Barrhead, Tofield, Edmonton (Crop Diversification Centre North (CDCN) and Ellerslie), Carstairs and Penhold, AB (Fig. 2-1). Fifty isolates collected from lupin by Dr. S.F. Hwang (Alberta Research Council, Vegreville) in 2005 were also included. These isolates were collected from lupin in 2005; the locations of the lupins were not recorded. All *Fusarium* cultures were subcultured from a single conidium or hyphal tip, and maintained on Spezieller

Nährstoffarmer Agar (SNA) (1.0 g KH_2PO_4, 1.0 g KNO_3, 0.5 g $MgSO_4 \cdot 7H_2O$, 0.5 g KCl, 0.2 g glucose, 0.2 g sucrose, and 20 g agar per 1 L water) at 4°C.

2.2.2.2 Identification of *Fusarium* isolates

Colonies were transferred from SNA to Carnation Leaf Agar (CLA) (Leslie and Summerell 2006) and PDA (BD/Difco, Sparks, MD) and incubated at room temperature under fluorescent light with a 12-hour photoperiod for identification. Observations of the cultural and microscopic characteristics of the isolates were recorded 10 to 14 days later. CLA plates of some isolates were kept for several months to check for slow forming chlamydospores, to aid in the differentiation of isolates suspected to be either *F. avenaceum* or *F. acuminatum*. Isolates were identified using the taxonomic key of Nelson et al. (1983), with modifications based on information contained within Leslie and Summerell (2006). The modifications included allowing the presence of polyphialides in cultures of *F. avenaceum* and the separation of *F. oxysporum* and *F. redolens* based on their morphology.

2.2.3 Screening for pathogenic *Fusarium* isolates

Pathogenicity tests were conducted to determine which isolates and species were pathogenic on lupin. The majority of isolates described in 2.2.2.1 were used in the pathogenicity screenings. As the culture collection from 2006 included multiple isolates from the same plant, isolates with very similar cultural morphology were excluded on the basis that they were probably the same species and possibly genetically identical.

The pathogenicity of the *Fusarium* isolates was investigated on the two *L. angustifolius* cultivars, Arabella and Rose, that are protected under the Plant Breeder's Rights Act in Canada and have been examined in agronomic experiments in Alberta.

Inoculum was produced by transferring isolates from SNA to PDA plates. The PDA plates were incubated at room temperature under cool fluorescent lights with a twelve-hour photoperiod for 13 to 15 days. The inoculum used consisted of a colonized PDA plate cut into pieces and homogenized with 60 mL of distilled water in a paper cup (Georgia-Pacific, Atlanta, GA).

Seeds of the cultivars Arabella and Rose were surface sterilized in 70% ethanol for 2 minutes, then in 0.6% sodium hypochlorite for two minutes and then rinsed in three changes of distilled water (Hwang et al. 1994) then air dried on paper towels placed on aluminium foil. Thirty-eight cell nursery trays (ITML Horticultural Products Inc, Brantford, ON, CA) were filled with pasteurized (121°C for 1hr) soil-less potting mix (ProMix BX , Premier Horticulture, Dorval, QC, CA). The potting mix was compressed with a studded tamp and five seeds of the same cultivar were placed in each cell. The inoculum of each isolate was poured over the seeds in ten cells, with five replicate cells for each cultivar. An uninoculated PDA plate served as the control. The treatments were arranged in a completely randomized design in a greenhouse. The seeds were then covered with ~1.5 cm of ProMix. A 12-hour photoperiod was provided by high-pressure sodium lights. The potting mix was watered as required with tap water and fertilized every two weeks with a 0.1% solution of 20N-20P-20K. Due to the large number of isolates, the first experiment was divided into two halves. The experiment was repeated with approximately twenty-five percent of the isolates, using the same methods.

Seedling emergence was assessed two weeks after the start of the experiment. Plants were examined weekly for signs of wilt, not associated with root rot, and scored on a zero to four scale (Salleh and Owen 1983). After five weeks, the plants were removed from their cells, washed free of potting mix and rated for root rot on a 0–4 scale (Hwang et al. 1994): 0 = healthy; 1 = small light brown lesions on <25% side of tap root; 2 = brown lesions on 25– 49% side of tap root; 3 = brown lesions on 50–74% side of tap root and tap root constricted; 4 = tap root extensively girdled and brown lesions on >75% side of tap root, limited lateral roots present, and plants wilted and stunted or dead. The plants were cut at the base of the shoot and the roots and shoots were placed in paper bags and weighed after drying for several days at ~40°C.

Attempts were made to reisolate *Fusarium* isolates that repeatedly generated root rot ratings ≥2. Isolates that were successfully reisolated were identified to species and used to inoculate seeds of Arabella and Rose using the above methodology.

2.2.4 Fusarium wilt pathogenicity assays

A series of small studies were conducted to attempt to determine if Fusarium wilt of lupin is present in Alberta. Isolates of *Fusarium* recovered from stem vascular tissue of cv. Arabella at CDCN were examined to determine if they were capable of causing wilt. *Fusarium oxysporum* f. sp. *lupini* race 3 (CBS 280.8 / IMI 141142), imported under permit from the Centraalbureau voor Schimmelcultures (CBS) in the Netherlands, was used as a positive control in the tests.

2.2.4.1 Root dip inoculations with cultivars Arabella and Rose

The root dip inoculation method was used to test the susceptibility of Arabella and Rose to *Fusarium oxysporum* isolates recovered from the stems of two lupin plants and to *F. avenaceum* recovered from the stem of one plant. The inoculum was a conidial suspension produced by scraping the surface of 11 day old cultures growing on PDA. Plants were inoculated using the root dip technique of Salleh and Owen (1983), and planted in thirty-eight cell nursery trays filled with autoclave Promix BX, with three plants per cell and eight cells per combination of treatment and cultivar; plants inoculated with sterile water served as the control. The plants were monitored for wilt using the scale of Salleh and Owen (1983). At the end of the experiment, the plants were examined for vascular discolouration and discoloured tissue plated on peptone pentachloronitrobenzene (PCNB) agar (Leslie and Summerell 2006) for reisolation.

2.2.4.2 Continuous dip inoculations

Due to the resistance of cultivars Rose and Arabella to Fusarium wilt and the lack of an available susceptible cultivar, 19 lupin lines (Table 2-5) were tested against *F. oxysporum* f. sp. *lupini* race 3 strain (CBS 280.8 / IMI 141142) to find a susceptible line. The experiment was based on the glass tube inoculation method of Rataj-Guranowska (1987). Seeds of all lupin varieties were surface sterilized (Robinson et al. 2000) and germinated in sterilized perlite (W.R. Grace & Co of Canada Ltd., Ajax, ON). *Fusarium oxysporum* f. sp. *lupini* was cultured on PDA for two weeks, then the surface of the colonies were scraped onto cheese cloth and washed with sdH_20, to produce a conidial suspension that was adjusted to 5×10^5 conidia mL^{-1}. Test tubes (18 mm diameter by 150 mm high) were filled 75% full with either spore suspension or sterile distilled water that served as the control. Four 6-day-old seedlings of the same variety were added to each tube and two tubes were used for each combination of variety and inoculation. Sterilized cotton was used to secure the plants in place. The tubes were arranged in a completely randomized design. The plants were monitored for two weeks, with water levels in the tubes topped up with sterile distilled water as required. Wilt was measured according to the scale of Salleh and Owen (1983).

The experiment was repeated using the only apparently wilt-susceptible lupin line, FBRF, and with the addition of three Alberta *F. oxysporum* isolates (216 and 351 from stem vascular tissue and 356 from root vascular tissue). Three replicate tubes were used per treatment, with only two seedlings per treatment. Tubes were arranged in a completely randomized design.

2.2.4.3 Long term pathogenicity test with lupin variety FBRF

The response of lupin variety FBRF to *F. oxysporum* isolates was examined using a standard root dip inoculation assay. Seeds of FBRF were sterilized according to Robinson et al. (2000) and sown in sterilized perlite (W.R. Grace & Co of Canada Ltd., Ajax, ON). After six days, seedlings were removed from perlite and their roots washed in tap water. Five *F. oxysporum* isolates (215, 216, and 217 from stem vascular tissue of the same plant, 351 from stem vascular tissue of a second lupin plant, and isolate 356 from lupin roots) were cultured on SNA and used to create conidial suspensions in sterile distilled water. Sterile water served as a control. Plants were inoculated using the method of Salleh and Owen (1983) and potted in autoclaved Promix BX in small plastic cups (450 mL tuffcups, Georgia-Pacific, Dixie Business, Norwalk, CT), with four plants per cup. There were six cups per treatment. Excess conidial suspension was applied to the potting mix in the pots. The cups were arranged in a completely randomized design. The plants were examined for signs of wilt and after 53 days the plants were scored for wilt, examined for signs of vascular discolouration and attempts were made to reisolate the fungus on PCNB agar if discolouration was present. The experiment was repeated once.

2.2.5 Statistical analysis

2.2.5.1 Pathogenicity screenings

All analyses were conducted using SAS software (version 9.1.3, SAS Institute Inc., Cary, NC). Data from all replicates were analyzed separately due to some isolates not being present in all replicates. The isolates were ranked by emergence, root rot severity and shoot weight and they did maintain the same rank order between runs when present in multiple replicates. All data was tested for normality using the Kolmogorov-Smirnov test. Shoot weight was examined by an analysis of variance (ANOVA) and Dunnett's t-test (one-tailed, treatment<control) to determine the pathogenicity of the isolates compared to the controls.

Emergence and root rot severity ratings were analyzed non-parametrically. The data were first ranked using PROC RANK in SAS and then analyzed using PROC MIXED according to the method of Shah and Madden (2004) to generate ANOVA type statistics (ATS). As there was no significant interaction between isolates and cultivars, the data from the two cultivars were pooled for analysis. Isolates were compared to the control by contrasts and adjustments for multiple comparisons were made using step-down Bonferroni adjustments in PROC MULTTEST to control type I error.

To determine if there were differences in the pathogenicity of isolates of *F. avenaceum*, the predominant pathogen, from different locations, shoot weight, emergence, and root rot were analyzed by PROC MIXED where *F. avenaceum* isolates were nested within location and the trials were treated as random effects. Tukey's Honestly Significant Difference (HSD) test at $p<0.05$ was used for means separation. The lettered groupings of differences in least squares means were produced using a macro (Saxton 1998).

2.2.5.2 Root dip inoculations with Arabella and Rose

The wilt severity rating of all plants in a cell was averaged and the average wilt severity rating compared using PROC GLM. Fusarium treatment means were compared to the control using Dunnett's t-test to determine if any treatments caused more severe wilt than the control.

2.2.5.3 Continuous dip inoculations

In the 19-variety glass tube test, the wilt scores of all plants in a tube were averaged and analyzed using PROC GLM. Means of the inoculated and uninoculated treatments for each variety were compared followed by step-down Bonferroni corrections to control type I error. In the second continuous dip test, the wilt scores of all plants in a tube were averaged and analyzed using PROC GLM. Treatments were compared using Tukey's HSD test.

2.2.5.4 Long term pathogenicity test with FBRF

Average wilt scores for plants in each pot for each treatment were analyzed using the Kruskal-Wallis test.

2.3 Results

2.3.1 Isolation and identification of possible fungal pathogens

In 2006, the fungi recovered from diseased roots (n=56) were (in order of isolation frequency): *Fusarium* spp. (71.4% of roots), *Rhizopus* spp. (37%), *Penicillium* spp. (19%), *Pythium* spp. (18%), *Botrytis* spp. (5.4%), *Rhizoctonia* spp. (3.6%) and *Alternaria* spp. (3.6%) (Table 2-1). The majority of roots had more than one fungal species recovered from them. For the roots colonized by *Fusarium spp.*, 42.5% of the roots were colonized by multiple *Fusarium* species, with up to six different species recovered per root. The most common species isolated from lupin roots were *F. avenaceum* (60%), *F. acuminatum* (30%), and *F. oxysporum* (30%) (Fig. 2-2). *Fusarium oxysporum* was also isolated from stem vascular tissue of two plants at CDCN in 2006 and *F. avenaceum* from one plant. The three plants were also suffering from root rot and had other *Fusarium* species colonizing their roots.

Examining the isolates from 2005 and 2006, it is evident that *F. avenaceum* is the predominant *Fusarium* species (36% of isolates) followed by *F. oxysporum* (20%) (Table 2-2). *Fusarium avenaceum* was the most commonly isolated *Fusarium* species at all locations in both years except for CDCN in 2005, where *F. oxysporum* was equally common, and Ellerslie in 2006 and Tofield in 2005, where *F. oxysporum* was slightly more common. Also, *F. avenaceum* was more widespread than other species being recovered from all locations. *Fusarium poae* and other members of the Section *Sporotrichiella* were only recovered at CDCN in 2005 and 2006.

2.3.2 Screening for pathogenic *Fusarium* isolates

In all, 285 isolates, 116 from surveys in 2005, 50 isolates (mostly *F. oxysporum*) from 2005, and 119 from surveys in 2006 were tested for pathogenicity towards lupin cultivars Arabella and Rose. In all experiments, *Fusarium* isolate and cultivar had a significant effect on dry shoot weight and seedling emergence (Table 2-3) and *Fusarium* isolate had a significant effect on the average root rot score. There was no significant interaction between *Fusarium* isolate and cultivar for any of the response variables measured. Seedling emergence and final shoot weight were consistently greater for cultivar Rose compared to Arabella, in all experiments. Significant reductions in shoot weight and emergence occurred only with isolates of *F. avenaceum* and one unidentified *Fusarium* isolate (Table 2-4). The majority of *F. avenaceum* isolates (50.6%) caused significant levels of root rot, followed by *F. acuminatum* (18.2%). The pathogenicity of *F. avenaceum* isolates was easily reproduced in subsequent experiments, whereas only one *F. acuminatum*, one *F. oxysporum*, and two unidentified isolates were consistently pathogenic. When isolates were grouped into discrete classes based on shoot weight, root rot severity, and emergence only isolates of *F. avenaceum* appeared in the highest classes (Fig. 2-3). For all other species, the majority of the isolates appeared in the lowest classes (Fig. 2-3).

Fusarium avenaceum was reisolated from severely diseased plants and when reinoculated onto lupin seeds the disease symptoms observed in the initial pathogenicity experiments were reproduced. Wilt symptoms were not observed on any plants other than ones inoculated with *F. avenaceum* and suffering from root rot (data not shown).

Analysis of the pathogenicity of *F. avenaceum* isolated from different locations showed that there were no significant effect of the origin of the strains on shoot dry mass (P=0.46), emergence (P=0.30), or root rot severity (P=0.29).

2.3.3 Fusarium wilt pathogenicity assays

2.3.3.1 Root dip inoculations with Arabella and Rose

No *Fusarium* isolates caused significantly more wilt than the control or were associated with any severe wilt symptoms. Vascular discolouration was only observed in one plant inoculated with *F. avenaceum*, but no fungus could be reisolated.

2.3.3.2 Continuous dip inoculations

Of the 19 lupin lines screened against *F. oxysporum* f. sp. *lupini* race 3, only line FBRF suffered severe wilt compared to the control (Table 2-5).

In the second continuous dip test using only FBRF, *F. oxysporum* f. sp. *lupini* again caused severe wilt or mortality (Table 2-6). Minor wilt symptoms did occur with one Alberta *F. oxysporum* isolate (216) recovered from lupin stem vascular tissue, but the isolate could not be reisolated from vascular tissue.

2.3.3.3 Long term pathogenicity test with FBRF

All plants survived to flower and set seed. No severe wilt symptoms occurred and the effect of inoculation was not significant. Vascular discolouration was minor and rare and no *Fusarium* could be recovered from the stems of any plants.

2.4 Discussion

In 2006, *Fusarium* spp. were the dominant species recovered from lupin roots (Table 2-1). This is similar to previous surveys of lupins in Alberta (Chang et al. 2005, 2006b). The other genera isolated in 2006 have also been previously isolated from lupins in the same area (Chang et al. 2005, 2006b). The detection of *Fusarium* as the dominant species among the mycoflora of lupin roots is a common occurrence. Surveys in Australia have found that *Fusarium* can be recovered from ~97% of diseased lupin roots (Sweetingham 1989). In parts of North America, *Fusarium* has been found to be commonly isolated from lupins (Weimer 1944; Kalis et al. 1990). With respect to identified *Fusarium spp.* in particular, *F. avenaceum* was the most commonly isolated species and the only species to be recovered at all locations, with *F. oxysporum* being the second most commonly recovered species (Table 2-2). The occurrence of *F. avenaceum* as the dominant *Fusarium* species from narrow-leafed lupin roots is not surprising. Kalis et al. (1990) found *F. avenaceum* was common on white lupin in Minnesota. *Fusarium avenaceum* is known as a root rot causing pathogen in Europe, but generally is not the dominant pathogen. Studies in Denmark and Belarus have found *F. oxysporum* and *F. solani* to be the most common *Fusarium* species (80%), with *F. avenaceum* being isolated only from young autumn sown plants (Danish Research Centre for Organic Food and Farming (DARCOF) 2005). *Fusarium avenaceum* has been reported as one of the most commonly recovered species from diseased white lupins in the U.K. during the winter (Bateman 1997). Conversely, Weimer (1944) found that *F. oxysporum* was the species most commonly recovered from *L. angustifolius* in the southeastern U.S.A. in multiple years and made no mention of *F. avenaceum*. This possibly resulted from the southern location of the study and the fact that *F. avenaceum* is most commonly found in more temperate areas. Australian surveys have also found *F. oxysporum, F. solani, F. acuminatum*, and *F. equiseti* to be the most commonly recovered species and made no mention of *F. avenaceum* (Sweetingham 1989).

The recovery of *F. acuminatum* from lupin roots is to be expected. *Fusarium acuminatum* is a common saprophyte in temperate areas and also a pathogen of legumes (Leslie and Summerell 2006). It has been reported to be a common colonizer of lupin roots in Australia (Sweetingham 1989) and was recovered from white lupin in America (Kalis et al. 1990). It is commonly found in basal tissues of diseased plants in Canada (Gordon 1956). *Fusarium spp.* identified as being in section *Discolor* were not identified to species due to similarities in morphology between the species of the section and the lack of pathogenic isolates in the section. Most isolates did appear to resemble *F. culmorum*. This species is often reported as being recovered from lupins at a low frequency (Sweetingham 1989; Bateman 1997; DARCOF 2005). Other isolates appeared to be *F. sambucinum, F. crookwellense, F. graminearum, or F. flocciferum*. *Fusarium flocciferum* has been recovered from lupins and is found in temperate areas (Brayford 1997), but no perithecia or sclerotia were observed, which makes it more improbable that the isolates were *F. flocciferum* or *F. graminearum*. Molecular techniques would have been necessary to reliably separate the species, although there are no species-specific primers for *F. flocciferum*. The rarity of members of the section *Liseola*, restricted to one isolate from Westlock, is of note. Members of the section, including *F. moniliforme sensu lato* have been previously reported as pathogens of narrow-leafed lupin in America (Weimer 1944), have been recovered from white lupin in the U.S.A. (Kalis et al. 1990), and have a worldwide distribution (Leslie and Summerell 2006).

Since fungal isolations were performed only from diseased lupins, it is unknown how the species that were recovered differ from the natural mycoflora of healthy lupin roots. A number of other factors may have also biased the isolation towards particular species and reduced the isolation of other species. Isolations were restricted to a single cultivar, Arabella. Arabella was bred to have resistance to root rot caused by *F. avenaceum* and is also resistant to wilt caused by *F. oxysporum* (Joernsgaard et al. 2004; DARCOF 2005; Kutpsov et al. 2006). It is possible that if isolations were made from lupins with different genotypes, a different pattern of species prevalence would have been found. The incidence of *Fusarium spp.* has also been reported to vary on resistant and susceptible cultivars of other crops (Lou et al. 1999).

The mycoflora of lupin roots may have been influenced by the use of seed treatments. Seed from 2005 and 2006 were treated with Apron Maxx (fludioxonil + metalaxyl-M) prior to seeding. The presence of fungicides may have favoured and deterred certain fungal species. Metalaxyl-M is a systemic fungicide with activity against Oomycetes and fludioxonil is a broad-spectrum contact fungicide with activity against *Fusarium* and other species. The use of Metalaxyl-M may have reduced the frequency of colonization by *Pythium*, an Oomycete. Certain *Fusarium* species have been reported to be more tolerant to fludioxonil on maize seeds than other species (Munkvold and O'Mara 2002) and sensitivity of isolates within a species can also vary (Broders et al. 2007). The relatively late collection and isolation of root fungi in 2006 may have also favoured the isolation of secondary invaders and saprophytes colonizing already diseased plants. In Denmark, recovery of *Fusarium* spp. from 8-week-old lupin roots resulted in more non-pathogenic species than did recovery at four weeks (DARCOF 2005).

Pathogenicity screenings showed that *F. avenaceum* is clearly the dominant *Fusarium* pathogen of lupin in central Alberta (Table 2-4). It was the most commonly recovered *Fusarium* spp. and caused the most severe disease symptoms (Figs. 2-2 and 2-3). The occurrence of *F. avenaceum* as a pathogen of narrow-leafed lupin is not an unusual occurrence. It has been reported as capable of causing severe seedling blight and root rot of lupins in both central and eastern Europe (Wollenweber and Reinking 1935; Schneider 1958; Debelyi et al. 1977). *Fusarium avenaceum* isolates demonstrated a wide range of pathogenicity towards lupins. This variation in pathogenicity amongst isolates of *F. avenaceum* is similar to results reported for *F. avenaceum* from lupins in Europe and from other pulse crops in Alberta. Schneider (1958) also found variations in the pathogenicity of *F. avenaceum* isolates recovered from *L. angustifolius*. The differences in pathogenicity of *F. avenaceum* isolates towards lupins have been found to range from non-pathogenic to isolates that can kill all hosts, including resistant cultivars (DARCOF 2005). Hwang (1994) found differences in root rot severity and shoot dry weight for lentils infected with different strains of *F. avenaceum* isolated within Alberta. Other than *F. avenaceum*, only two isolates were able to cause a significant reduction in shoot weight or emergence (Table 2-4). One of these was most likely a degenerate *F. avenaceum* isolate, but conclusive identification based on morphology was not possible.

The lack of any severe root rot or seedling blight symptoms occurring with the inoculation of *F. oxysporum*, *F. redolens*, and *F. solani* contrasts with previous results from central and eastern European and southern U.S.A. studies where members of the species are highly aggressive against narrow-leafed lupin (Weimer 1944; Debelyi et al. 1977; DARCOF 2005). The results are more similar to Australian findings where *F. oxysporum* and *F. solani*, although common, were found to be either weakly pathogenic or non-pathogenic (Sweetingham 1989). In western Europe, *F. avenaceum* has also been found to be the dominant *Fusarium* pathogen on white lupin, with *F. oxysporum* being only weakly pathogenic (Bateman 1997).

Fusarium acuminatum, although capable of causing significant levels of root rot on lupin, was restricted to low to moderate root rot levels. *Fusarium acuminatum* is associated with diseased lupins (Sweetingham 1989; Kalis et al. 1990), but is usually not reported as a pathogen of lupins, although it is pathogenic to other legume species (Hancock 1983; Hwang et al. 1994). *Fusarium acuminatum* has been found to cause significant levels of root rot to lentil in Alberta (Hwang et al. 1994) but, as is the case with lupin, not to the same extent as *F. avenaceum*.

There was a lack of significant difference between any of the response variables measured and the origin of the *F. avenaceum* isolates.

The lack of any statistically significant differences for root rot severity ratings between the two cultivars Arabella and Rose, is not unexpected as both cultivars are the results of European breeding efforts to incorporate genes for resistance to *F. avenaceum* (Kutpsov et al. 2006). This is similar to the results for field trials in Alberta with cultivars Arabella and Rose and *F. avenaceum* (Chang et al. 2006). This study did not test the response to Alberta *Fusarium* pathogen populations of other European cultivars that are more susceptible to root rot or Australian cultivars that have been bred where *Fusarium* disease pressure is not problematic. It is possible that *F. avenaceum* maybe more of a hindrance to lupin cultivation if producers attempt to use other cultivars than the two tested here.

Whether or not the distribution of *Fusarium* species and isolation of *Fusarium* was a reflection of the species composition within soil is unknown, as no attempts were made to assess *Fusarium* spp. diversity within the field soil used to cultivate the lupins. The relatively high rate of recovery of *F. oxysporum* may have resulted in part from the natural tendency of the species to be present in soil at a higher frequency than most other *Fusarium* species (Gordon 1954; Gordon 1956; Kommedahl et al. 1988). Gordon (1956) found *F. oxysporum* represented 31% of *Fusarium* isolates recovered from Canadian soil samples, whereas *F. acuminatum* accounted for only 3% of isolates and *F. avenaceum* for less than 0.1%. In soils cropped to cereals, Gordon (1954) found the difference in the frequency between *F. oxysporum* and *F. avenaceum* to be greater.

The lack of any wilt caused by *F. oxysporum* or *F. redolens* is not particularly surprising and there are two possible explanations: either there was no Fusarium wilt to be found in Alberta or it was too rare or incapable of causing disease under the conditions tested. Unlike Fusarium wilts of other legume species such as pea, lentil, and chickpea that have a worldwide distribution (Infantino et al. 2006), Fusarium wilt of narrow-leafed lupin has only been found in Europe, particularly central and eastern Europe. Despite decades of cultivation of wilt-susceptible lupin cultivars over a wide area in Western Australia, often in a 1:1 continuous rotation, no Fusarium wilt has been reported, despite ongoing monitoring. In America, lupins were grown for three decades, reaching over one million hectares annually (Putnam 1993), without any reports of Fusarium wilt problems, despite the susceptibility of the cultivars being grown (Armstrong and Armstrong 1964). Moreover, narrow-leafed lupin has also been shown to only be susceptible to members of *F. oxysporum* f. sp. *lupini* race 3 from Europe (Armstrong and Armstrong 1964). This makes it unlikely that any Canadian isolates would have been capable of causing wilt.

It is possible, however, that if wilt of lupin was present in central Alberta, it was not detected. Isolations in 2005 and 2006 were performed only on the resistant cultivar Arabella. If isolations had been performed on a cultivar that was highly susceptible such as Prima (Kutpsov et al. 2006), it may have been more likely that *F. oxysporum* causing wilt would have been isolated, if present. The isolations from roots may have also overemphasized the importance of *F. oxysporum* in early surveys, as saprophytic and root-rot causing strains are also common colonizers of roots. It is possible that the *F. oxysporum* isolated from stem tissue of two plants may have represented non-pathogenic endophytic strains. The roots of both of these plants were infected with multiple *Fusarium* spp., including *F. avenaceum*, and any disease symptoms seen in the field could be attributed to root rot.

Despite the apparent lack of Fusarium wilt of lupin in Canada, it is possible that the disease could arrive from Europe with imported seeds and eventually spread to become a serious problem, if lupin cultivation continues. This is thought to be possible for other lupin growing areas (Department of Agriculture Government of Western Australia 2004). If lupin is adopted as a crop plant in central Alberta, it is clear that management strategies for the control of *Fusarium* and other root rot pathogens would be required.

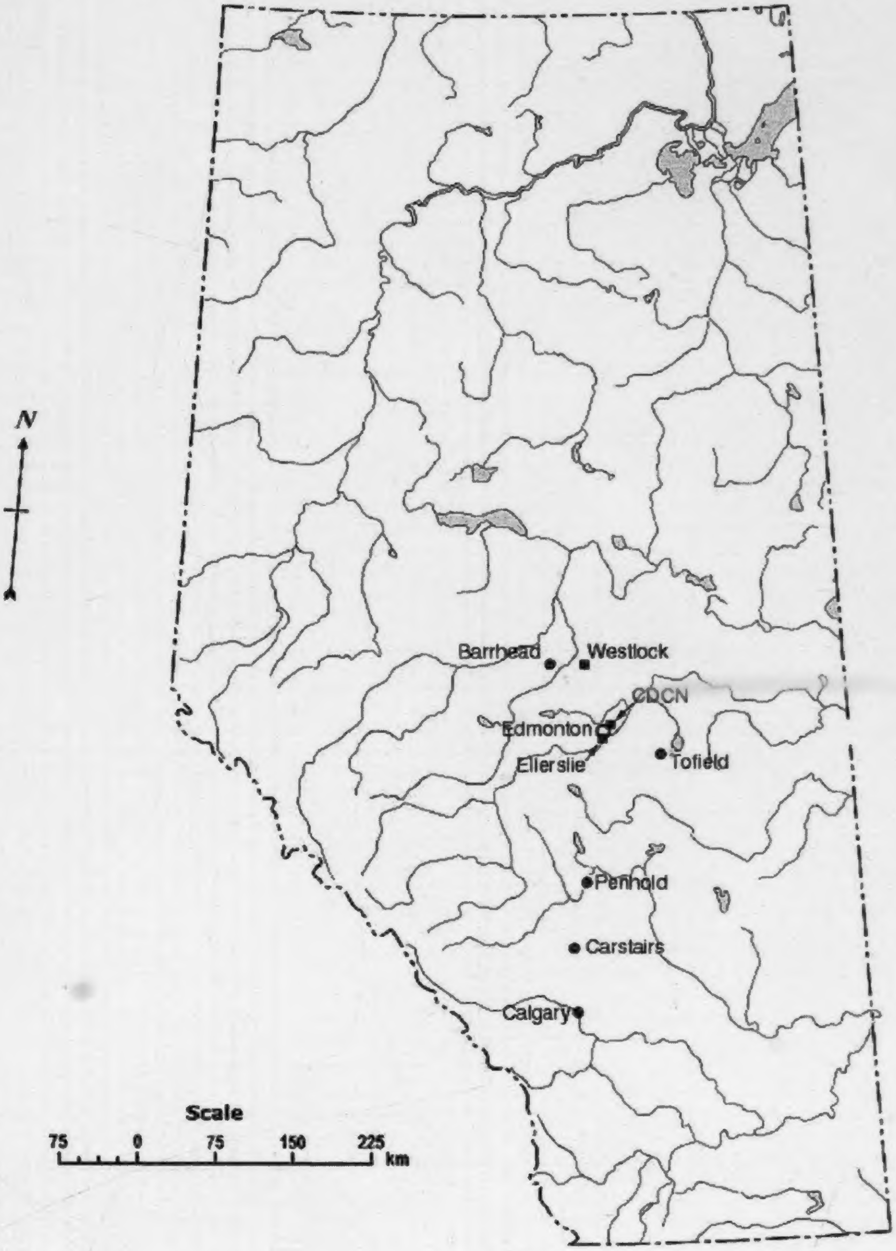

N
Barrhead
Westlock
CDCN
Edmonton
Ellerslie
Tofield
Penhold
Carstairs
Calgary
Scale
75
0
75
150
225
km

Figure 2-1: Location of lupin plots where *Fusarium* isolates were collected.

●: Sampled in 2005 ■: Sampled in 2005 and 2006

Table 2-1. Frequency of fungi isolated from taproot samples collected from experimental plantings of lupin in central Alberta in 2006.

Field Location	No. of roots sampled	Incidence of microorganisms (%)						
		Fusarium spp.	*Rhizopus* spp.	*Penicillium* spp.	*Pythium* spp.	*Botrytis* spp.	*Rhizoctonia* spp.	*Alternaria* spp.
CDCN	45	69	33	9	22	7	2	2
Ellerslie	5	100	40	80	0	0	20	0
Westlock	6	67	67	50	0	0	0	17
All locations	56	71	38	20	20	5	4	4

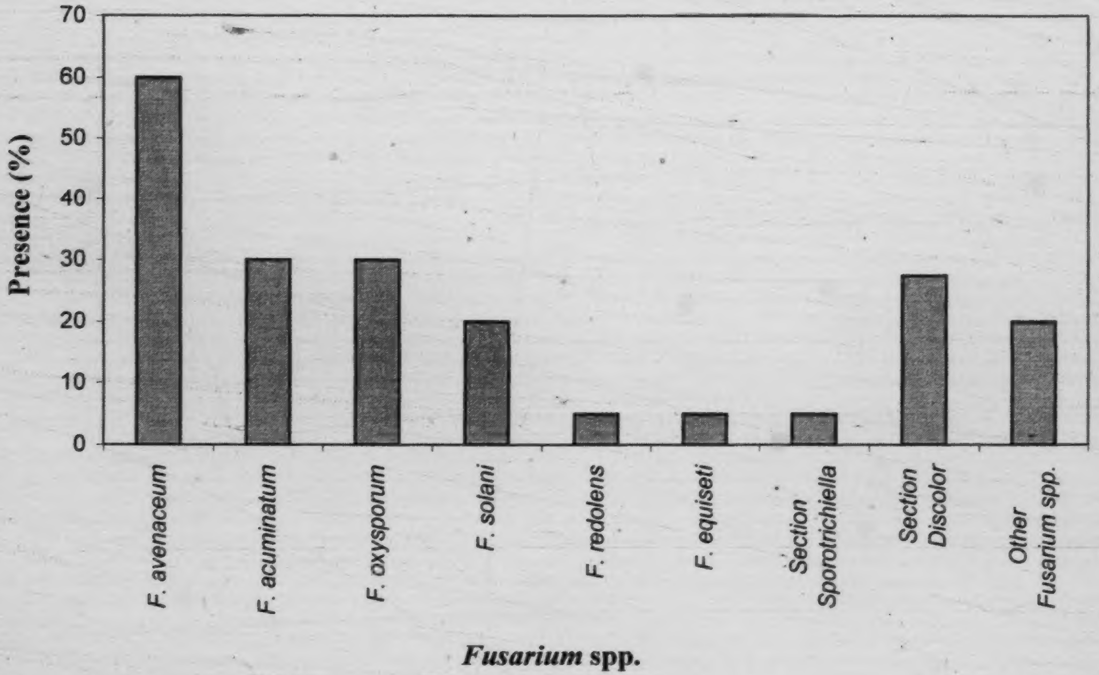


Figure 2-2: Recovery of *Fusarium* spp. from lupin roots in 2006.

Section *Sporotrichella* includes *F. chlamydosporum*, *F. poae*, *F. sporotrichioides*, and *F. tricinctum*, Section *Discolor* includes *F. crookwellense*, *F. culmorum*. *F. graminearum*, and *F. sambucinum*.

Table 2-2. *Fusarium* spp. isolated from lupin roots collected from experimental plantings of lupin in central Alberta in 2005 and 2006.

	Barrhead 2005	Carstairs 2005	CDCN 2005	CDCN 2006	Ellerslie 2005	Ellerslie 2006	Penhold 2005	Tofield 2005	Westlock 2005	Westlock 2006	Total
F. acuminatum	1	2	2	12	0	0	1	0	1	5	24
F. avenaceum	20	15	8	25	6	4	2	2	0	5	87
F. equiseti	2	0	0	0	0	0	0	0	1	2	5
F. oxysporum	10	5	8	17	0	5	0	3	0	2	50
F. redolens	1	0	1	1	0	0	0	0	0	1	4
F. solani	3	0	3	20	0	0	0	2	1	0	29
Section *Discolor*[a]	2	1	3	9	0	0	0	0	0	4	19
Section *Liseola*[b]	0	0	0	0	0	0	0	0	1	0	1
Section *Sporotrichiella*[c]	0	0	1	3	0	0	0	0	0	0	4
Other Fusaria	3	2	2	11	0	0	0	1	0	2	21
Total	42	25	28	98	6	9	3	8	4	21	244

[a] Section *Discolor* includes *F. crookwellense*, *F. culmorum*. *F. graminearum*, and *F. sambucinum*.

[b] Section *Liseola* includes *F. moniliforme sensu lato*.

[c] Section *Sporotrichella* includes *F. chlamydosporum*, *F. poae*, *F. sporotrichioides*, and *F. tricinctum*.

Table 2-3. Test statistics for the effects of *Fusarium* isolate and cultivar on emergence, root rot severity, and shoot weight of lupin.

Trial	Effect	Analysis of variance-type statistics (ATS)[a]								ANOVA statistics		
		Emergence				Root Rot				Shoot Weight		
		df_N	df_D	F	*P* value	df_N	df_D	F	*P* value	Df	F	*P* value
1	Isolate (I)	118	856	2.98	<0.0001	112	697	3.43	<0.0001	140	3.9	<0.0001
	Cultivar (C)	1	856	78.43	<0.0001	1	697	1.71	0.191	1	88.74	<0.0001
	C x I	118	856	1.07	0.296	112	697	1.21	0.0669	140	0.9	0.782
2	Isolate (I)	124	879	2.51	<0.0001	124	862	2.54	<0.0001	147	2.68	<0.0001
	Cultivar (C)	1	879	102.97	<0.0001	1	862	0.31	0.576	1	183.66	<0.0001
	C x I	124	879	1.1	0.221	124	862	0.92	0.724	147	1.21	0.0508
3	Isolate (I)	57.3	402	5.81	<0.0001	60.8	418	2.13	<0.0001	73	4	<0.0001
	Cultivar (C)	1	403	7.16	0.0075	1	418	0	0.989	1	44.96	<0.0001
	C x I	57.3	403	0.69	0.965	60.8	418	0.76	0.916	73	1	0.474

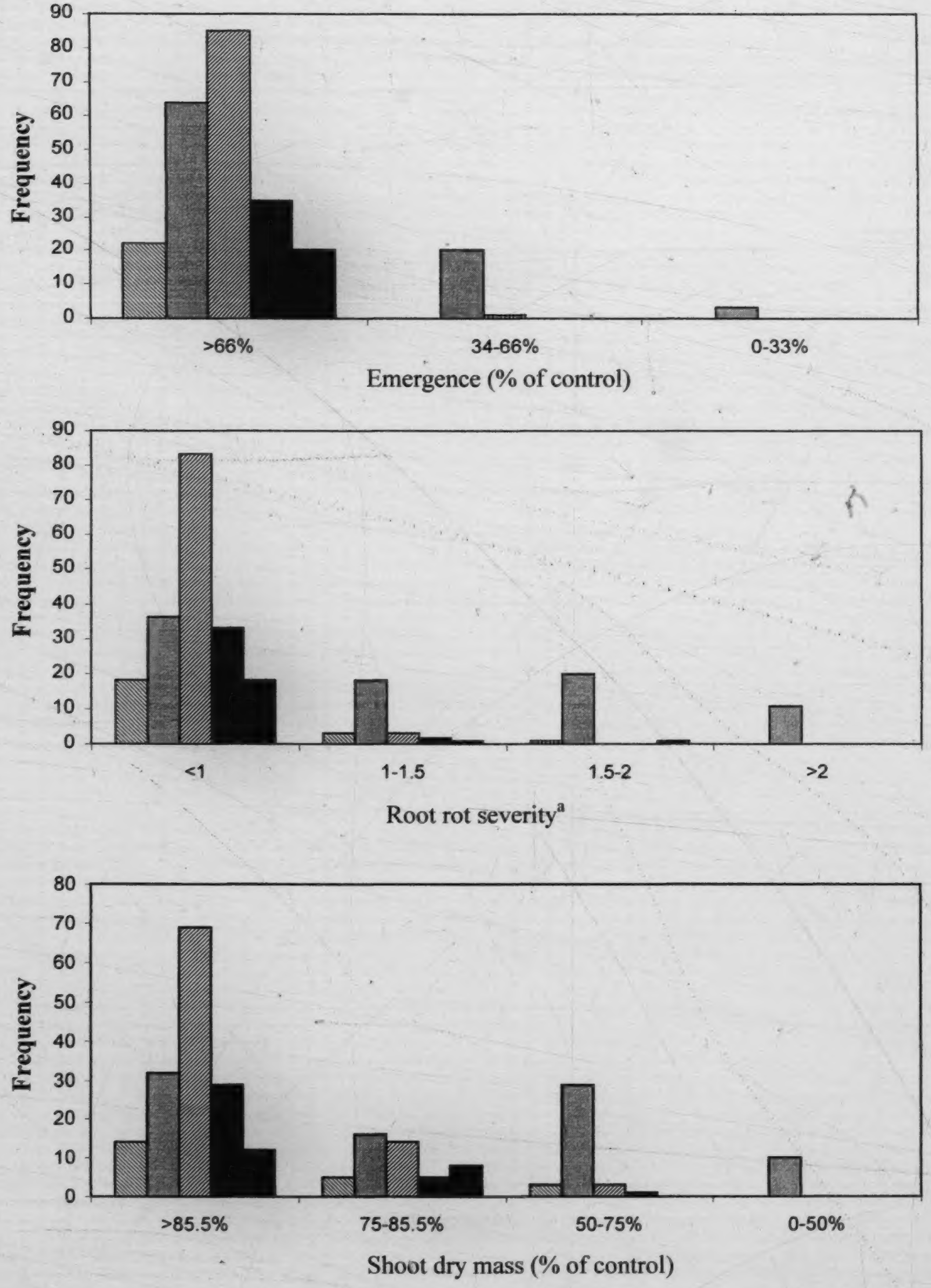
[a] Abbreviations: df_N = numerator degrees of freedom and df_D = denominator degrees of freedom.

Table 2-4. Pathogenicity of *Fusarium* isolates towards lupin seedlings.

Fusarium Species / Section	Number of Isolates Tested	Percentage (#) of Isolates adversely affecting Shoot Dry Weight	Emergence[a]	Root Rot	Percentage (Total #) of Pathogenic Isolates[b]
F. acuminatum	22	0 (0)	0 (0)	18 (4)	18 (4)
F. avenaceum	87	22(19)	14 (12)	51 (44)	56 (49)
F. equiseti	6	0 (0)	0 (0)	0 (0)	0 (0)
F. oxysporum	86	0 (0)	0 (0)	3.5 (3)	3.5 (3)
F. redolens	6	0 (0)	0 (0)	0 (0)	0 (0)
F. solani	35	0 (0)	0 (0)	2.9 (1)	2.9 (1)
Section *Discolor*	20	0 (0)	0 (0)	10 (2)	10 (2)
Section *Liseola*	1	0 (0)	0 (0)	0 (0)	0 (0)
Section *Sporotrichiella*	4	0 (0)	0 (0)	0 (0)	0 (0)
Other / Unidentified	18	5.6 (1)	5.6 (1)	11 (2)	17 (3)
Total	285	7.02 (20)	4.56 (13)	20.4 (58)	22.5 (64)

[a] Emergence recorded two weeks after harvesting.

[b] Isolates that were pathogenic for one or more of the response variables that were analyzed.


Frequency
90
80
70
60
50
40
30
20
10
0
>66%
34-66%
0-33%
Emergence (% of control)
Frequency
90
80
70
60
50
40
30
20
10
0
<1
1-1.5
1.5-2
>2
Root rot severity[a]
Frequency
80
70
60
50
40
30
20
10
0
>85.5%
75-85.5%
50-75%
0-50%
Shoot dry mass (% of control)

(continued)

Figure 2-3. Frequency of common *Fusarium* spp. grouped according to their effect on lupin emergence, root rot, and shoot dry mass.

▧ *F. acuminatum*, ▒ *F. avenaceum*, ▨ *F. oxysporum*, ▦ *F. solani*, ■ Section *Discolor*.

[a] Root rot rated using a 0–4 scale (Hwang et al. 1994): 0 = healthy; 1 = small lesions on <25% of tap root; 2 = lesions on 25–49% of tap root; 3 = lesions on 50–74% of tap root and tap root constricted; 4 = tap root extensively girdled and lesions on >75% of tap root, limited lateral roots present, and plants wilted and stunted or dead.

Table 2-5. Wilt severity for different lupin varieties inoculated with *F. oxysporum* f. sp. *lupini* race 3 isolate CBS 280.8 / IMI 141142, in a continuous dip assay.

	Wilt Severity (0-4)[a]	
Variety	Inoculated	Uninoculated
Arabella	0.25	0.38 ns
E8	0.25	0.25 ns
FBRF	3.13	0.25 ***
G107	0.13	0.25 ns
G11-7	0.25	0.38 ns
G12-12	0.13	0.0 ns
G22-1	0.38	0.0 ns
G32	0.88	1.0 ns
G851	0.00	0.88 ns
GA65	0.75	1.4 ns
MLU 317	0.25	0.63 ns
MLU-122	0.75	0.38 ns
MLU-318-1	0.25	0.0 ns
MLU-320	0.75	0.38 ns
MLU-324	0.88	1.0 ns
P12-1	0.38	0.88 ns
P53	0.50	0.50 ns
Rose	0.13	0.13 ns
W12-W5	1.13	1.0 ns

[a] Note: Number of observations per mean, $N = 2$, each observation is the average of four plants. The differences between non-inoculated and inoculated were significant for values followed by an asterisk, according to LSD test, with step-down Bonferroni adjustments ($P \leq 0.05$).

[a] Rated on visible wilt symptoms based on a 0–4 scale (Salleh and Owen 1983): 0 = healthy; 1 = temporary symptoms; 2 = slight wilting; 3 = wilt, defoliation up to 75% ; 4 = defoliation of more than 75%, dead.

Table 2-6. Wilt severity of lupin variety FBRF inoculated with different *F. oxysporum* isolates.

Isolate[a]	Wilt Severity (0-4)[b]
Control	0
F. oxy R3	3.5
F. oxy V1	1.3
F. oxy V2	0
F. oxy Rt1	0.17

Note: Number of observations per mean, $N = 3$, each observation is the average of two plants.

[a] Isolate: Control=sterile water; F. oxy R3= *F. oxysporum* f. sp. *lupini* race 3 isolate CBS 280.8 / IMI 141142; F. oxy V1= *F. oxysporum* from lupin stem vascular tissue , isolate 216; F. oxy V2= *F. oxysporum* from lupin stem vascular tissue, isolate 351 F. oxy Rt1 = *F. oxysporum* from lupin root, isolate 356;

[b] Rated on visible wilt symptoms based on a 0–4 scale (Salleh and Owen 1983): 0 = healthy; 1 = temporary symptoms; 2 = slight wilting; 3 = wilt, defoliation up to 75% ; 4 = defoliation of more than 75%, dead.

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3. Genetic diversity and possibility of recombination in *Fusarium avenaceum* isolated from lupins in central Alberta

3.1 Introduction

Fusarium avenaceum (Fr.) Sacc. (Teleomorph: *Gibberella avenacea* Cook) is a filamentous ascomycete. The species has a very wide host range and causes root rots, seedling blight, fruit rots, and head blight of cereals. It is cosmopolitan, although it is more frequently found in temperate areas. *Fusarium avenaceum* has long been recognized as being extremely variable morphologically (Schneider 1958). In recent years, the development of molecular techniques has facilitated the study of the genetic diversity and variation within *F. avenaceum*. European studies have shown the species to be very diverse genetically (Turner et al. 1998; Chełkowski et al. 1999; Satyaparasad et al. 2000), even on small spatial scales such as individual fields (Nicholson et al. 1993). Fewer studies have examined genetic variation within *F. avenaceum* outside of Europe. Benyon et al. (2000) found high levels of genetic variability in Australian *F. avenaceum* isolates and Nalim (2004) reported similar results among American isolates.

Fusaria, like most other filamentous ascomycete fungi, have a single regulatory locus (mating type (*MAT*) locus) that controls sexual reproduction (Nelson 1996; Coppin et al. 1997; Kerényi et al. 2004). Two different idiomorphs of the mating type locus exist, *MAT1-1* and *MAT1-2* (abbreviated *MAT-1* and *MAT-2*, respectively). The alternate sequences at the *MAT* locus are referred to as idiomorphs, rather than alleles, because they lack significant sequence similarity (Turgeon 1998). In Fusaria with a *Gibberella* teleomorph, the *MAT-1* locus contains three genes *MAT1-1-1*, *MAT1-1-2*, and *MAT 1-1-3* and the *MAT-2* locus contains one gene, *MAT1-2-1* (Yun et al. 2000). *MAT1-1-1* and *MAT1-2-1* encode transcriptional regulators with conserved DNA binding motifs. These genes contain a conserved α box domain and a high-mobility-group (HMG) box domain (Yun et al. 2000; Kerényi et al. 2004), which are believed to control the mating process and sexual development (Coppin et al. 1997; Yun et al. 2000). It is hypothesised that *MAT1-1-2* and *MAT1-1-3* are involved in ascosporogenesis (Yun et al. 2000; Kerényi and Hornok 2002). *Gibberella zeae* Schwein. (Petch) is the only *Gibberella* species identified as homothallic and contains both *MAT* loci (Yun et al., 2000). Heterothallic species contain only *MAT-1* or *MAT-2* and as such are not self-fertile.

Traditionally, mating type was determined by crossing isolates with MAT-tester strains (Mansuetus et al. 1997; O'Donnell et al. 1998). The technique is limited to only species that have a known sexual stage, which is inducible under laboratory conditions. With the development of diagnostic primers for *Gibberella* mating types by Kerényi et al. (2004), the identification of mating types in numerous *Fusarium/Gibberella* species is now possible. This has led to an increased ability to determine if *Fusarium* populations are of clonal origin or if the possibility of a cryptic sexual cycle exists within a species. The use of these methods has demonstrated that *F. avenaceum* is heterothallic and analysis of isolates from European culture collections has shown that both mating-types exist within Europe (Kerényi et al. 2004), but their prevalence and distribution within a natural population has not yet been determined.

In many *Fusarium* and other fungal species where sexual reproduction is observed rarely or not at all, several methods are available to determine if recombination and sexual reproduction occurs within populations. Strictly clonal populations should be characterized by widespread identical genotypes and low genetic diversity, as new genotypes arise only from mutation or immigration of new genotypes. There is also a strong association between alleles at different loci in asexually reproducing populations leading to linkage disequilibrium. In clonal reproduction, the entire genome of a organism is effectively linked (Anderson and Kohn 1995). In randomly mating (panmictic) populations, recombinant genotypes are produced that promote and maintain genotypic diversity. Recombination also leads to the random association of alleles at different loci, causing linkage equilibrium. The more random mating that occurs in a population compared to asexual or non-random mating, the less the linkage disequilibrium. In heterothallic species that are randomly mating, the mating type ratio should approach 1:1 (Milgroom 1996; Pever et al. 2004). Most fungal populations exist somewhere between the two extremes of clonality and panmixis and species show characteristics of both reproductive modes.

The primary objectives of the present study were to examine the population structure of *F. avenaceum* isolated from *L. angustifolius* in Alberta with molecular markers, and to identify the mating types of the *F. avenaceum* isolates examined, comparing this information to geographic origin and pathogenicity.

3.2 Materials and methods

3.2.1 Isolates

Fifty *Fusarium* isolates selected from the collections in 2005 and 2006, used in chapter 2, were selected for use in this study, including forty-one isolates putatively identified as *F. avenaceum*, six *F. acuminatium* isolates, two unidentified isolates believed to belong to the section *Gibbosum*, and one isolate of *F. oxysporum* included as a phenotypic outlier. *Fusarium avenaceum* isolates were selected to include those from all locations sampled and to include isolates showing differing aggressiveness towards lupin.

3.2.2 DNA extraction

Isolates were cultured in flasks containing 50mL of potato dextrose broth (PDB) (BD/Difco, Sparks, MD) for seven days, and then rinsed with two volumes of sdH_2O. DNA was extracted from the mycelium using the CTAB protocol of DePriest et al. (2005) with RNase treatment.

The quality of the purified DNA was tested by amplification with primers ITS 4 and 5 (White et al. 1990). DNA concentration was quantified on a Nanodrop Spectrophotometer ND-1000 (Thermo Fisher Scientific, Waltham, MA) prior to use.

3.2.3 RAPD and MP-PCR analysis

Two decamer (RAPD) primers and three microsatellite primers (MP) (Table 3-1) were used for PCR amplification. Genomic DNA was diluted to 5ng μL^{-1} in TE. Amplifications were performed using a GeneAmp PCR System 9700 Thermocycler (Applied Biosystems, Foster City, CA) in a 25 μL reaction volume containing 25 ng *Fusarium* DNA , 1X GoTaq Green Master Mix (Promega, Madison, WI), and 0.5 µM primer. The reaction conditions of Balmas et al. (2005), with minor modifications, were used. These consisted of initial denaturation at 94°C for 2 min, followed by 40 cycles of 1 min denaturation at 94°C, 1 min annealing at 37°C for RAPD primers or 50°C for microsatellite primers, and a 2 min extension at 72°C followed by a final elongation step of 10 min for RAPD primers or 7 min for MP primers, then a 4 °C hold. Negative controls included all reagents with sdH_2O substituted for DNA. All reactions were repeated once. Amplification products were separated on 1% agarose gels containing ethidium bromide (0.2 μg mL^{-1}) in TAE buffer for 120 min at 2.27 V cm^{-1}. Gel images were acquired with a Flourochem SP system (Alpha Innotech Corp., San Leandro, CA).

Amplification products were manually scored as "1" for present and "0" for absent. Only reproducible fragments were scored. Genetic similarities were calculated for the RAPD, MP-PCR, and combined RAPD and MP-PCR data sets in RAPDistance v1.04 (Armstrong et al. 1996) using the method of Nei and Li (1979). The Nei-Li coefficient is defined as $d_{ij}=2a/(2a+b+c)$ where d_{ij}= Nei-Li coefficient, a=number of 1-1 matches, b=number of 1-0 matches, and c=number of 0-1 matches. The RAPD and MP-PCR matrixes were compared using the Mantel test with 1000 permutations in Pop Tools 2.75 (www.cse.csiro.au/CDG/poptools). The similarity coefficients were used to construct a dendrogram using the unweighted pair group method of arithmetic means (UPGMA) with the NEIGHBOR program of PHYLIP 3.66 (Felsenstein1993). Support for the reliability of the UPGMA clusters was determined by subjecting the binary data to bootstrap analysis with 1000 replications using the program WINBOOT (Yap and Nelson 1996).

To determine the possibility of recombination within *F. avenaceum* populations, the Index of Association (I_A) test for multilocus genetic disequilibrium was employed (Brown et al. 1980). The number of heterozygous loci between individuals (k) within a population was calculated. The variance of k (V_d) was also calculated and compared to the variance expected (V_e) under linkage equilibrium. The null hypothesis of linkage equilibrium H_0 $V_d= V_e$ was tested by Monte Carlo simulation with 1000 resamplings. The analysis was performed using the LIAN 3.5 program (Haubold and Hudson 2000). The I_A is a value of the observed variance compared to the expected variance ($I_A = V_D / V_E - 1$), and has an expected value of zero under random mating (linkage equilibrium). Because the I_A increases with the number of loci analyzed, the standardized index of association ($I^S_A= I_A / (r- 1)$), where r is the number of loci analyzed, was also calculated to compare populations with different numbers of loci (Hudson 1994; Haubold and Hudson 2000).

3.2.4 *Fusarium avenaceum* mating-type idiomorph analysis

All *F. avenaceum* isolates analyzed for genetic diversity were examined using the semidegenerate primers of Kerényi et al. 2004 (Table 3-2) to determine their mating type. Amplification was performed in a 10μL reaction volume consisting of 1x GoTaq Green master mix (Promega, Madison, WI), 0.25μM of each forward and reverse primer, and 20ng fungal DNA. The PCR conditions consisted of an initial denaturation at 94°C for 2 min, followed by 35 cycles of 30 s denaturation at 94°C, 30 s annealing, and 30 s extension at 72°C followed by a final elongation step of 5 min. The annealing temperatures were 55°C for fusALPHAfor/fusALPHArev and 60°C for fusHMGfor/fusHMGrev. The negative control included the PCR reagents and sterile water instead of DNA, DNA from the homothallic species *F. graminearum* was used in the positive control. Amplification products were separated on 1% agarose gels containing ethidium bromide (0.2 μg mL^{-1}) in TAE buffer running for 50 min at 3 V cm^{-1}. Gel images were acquired with a Flourochem SP system (Alpha Innotech Corp.).

3.2.5 Relationship between genetic diversity, mating type, and pathogenicity

The pathogenicity of the different UPGMA clusters and mating types of *F. avenaceum* were compared using a nested factorial design with data from Chapter 2, using shoot weight as the response variable. Cultivar, UPGMA group, and mating type were treated as fixed effects, *F. avenaceum* isolates were nested within UPGMA group and mating type, and replicate trials were treated as random effects.

3.3 Results

3.3.1 RAPD and MP-PCR analysis

Forty reproducible and polymorphic markers were obtained. The genetic distances produced by the RAPD and MP-PCR markers were highly correlated (R^2=0.70; *P* by Mantel test was <0.001) and the combined dataset was used for further analysis. All isolates had a unique multilocus haplotype, giving a genotypic diversity of 100%. The dendrogram based on genetic similarity of the isolates is shown in Fig 3-1. The *F. avenaceum* isolates formed two large, equally sized clusters (arbitrarily named Group I and Group II) with strong bootstrap support (87.7%) (Fig. 3-1). All other *Fusarium* isolates fell into other distinct clusters or were located individually on the dendrogram. Isolate 371, which was initially identified as *F. acuminatum*, clustered amongst the *F. avenaceum* isolates of Group I. Upon re-examination of the morphology of that isolate, it was classified as *F. avenaceum*. Isolates within *F. avenaceum* Group I showed a genetic similarity of 90.3% or greater, isolates within Group II had a genetic similarity of at least 88.3%. The similarity between the two *F. avenaceum* clusters was 86.78%. Within Group I, *F. avenaceum* isolates 253 and 213 shared a genetic similarity of 98.65%, but were differentiated by strong bootstrap support (79.1%). There was no correlation between the isolates' origins and their grouping within the dendrogram.

3.3.2 *Fusarium avenaceum* mating-type idiomorph analysis

All *F. avenaceum* isolates carried one mating type gene (Fig. 3-1) and both mating types were identified. Twenty-five isolates were typed as *Mat-1* and 17 as *Mat-2*. Both mating types were present in all locations examined, except for Penhold, where both isolates were *Mat-2*. The mating type ratios (*Mat-1*:*Mat-2*) were 10:11 and 15:6 in UPGMA Group I and II, respectively, and 25:17 overall.

3.3.3 Analysis of recombination within *F. avenaceum* populations

Linkage equilibrium tests were performed on *F. avenaceum* isolates from each UPGMA group, both UPGMA groups combined, and each location, when enough isolates were available. The I_A, I^S_A, and *P* values are shown in Table 3-3. The null hypothesis of linkage equilibrium could not be rejected for any of the populations of Group I or for all Group I isolates combined, indicating that Group I isolates form a randomly mating population. There was more variation in the rejection of the null hypothesis for populations of Group II isolates and for locations where group I and Group II isolates were combined. The null hypothesis was not rejected for Group II isolates from Barrhead or CDCN, but was rejected for Group II isolates from Ellerslie and all Group II isolates combined. The null hypothesis could not be rejected for all *F.*

avenaceum isolates (groups I and II combined) from Carstairs and Westlock, but could be rejected for all other locations.

3.3.4 Relation of genetic diversity and mating type to pathogenicity.

Analysis of variance revealed no significant effect (P>0.05) of UPGMA group or mating type or any interaction between any of the factors on shoot weight.

3.4 Discussion

This work represents the first attempt to characterize a large number of Canadian *F. avenaceum* isolates with molecular methods. Using random markers a high level of genotypic diversity was detected amongst *Fusarium* isolates and each isolate had a unique haplotype. There was no apparent relation between the origin of the *F. avenaceum* isolates and the clustering of the isolates, nor was there any relation between clustering and pathogenicity (Fig. 3-1). The *F. avenaceum* isolates shared high genetic similarity values (>86%), but exhibited 100% genotypic diversity. The results of previous RAPD studies examining the genetic similarity amongst *F. avenaceum* isolates have varied. Yli-Mattila et al. (1996) found that the similarity between *F. avenaceum* isolates from within Finland could be below 30%. Conversely, Turner et al. (1998) generated 157 RAPD markers and found that similarity between 17 *F. avenaceum* isolates from three European countries was ~85% or more, which is similar to the values presented here.

Fusarium avenaceum is known to be highly diverse genetically as well as morphologically. These results are comparable to RAPD analysis of European *F. avenaceum* populations where extremely high genotypic diversity was found to be the norm and hierarchal clustering showed little relation to origin or pathogenicity. Satyaparasad et al. (2000) found 67 haplotypes among 68 *F. avenaceum* isolates recovered mainly from *L. albus* in the United Kingdom, and no correlation between RAPD grouping and pathogenicity to lupins. Isolates also did not consistently group according to their host plant or geographic location. Similar results have been found in northern Europe, where Yli-Mattila et al. (1996) analyzed 33 *F. avenaceum* isolates from cereal roots and stem bases using RAPD and found that all possessed unique haplotypes, with no relationship between RAPD profile and geographic origin. *Fusarium avenaceum* from cereal samples in the Netherlands have been shown to be highly diverse, with 13 haplotypes among 17 isolates when only three RAPD primers were used (De Nijs et al. 1997). When secondary metabolite (SM) profiles were added to RAPD profiles, all isolates could be distinguished. Analysis of central European *F. avenaceum* populations have also shown *F. avenaceum* to be highly diverse within Poland (Chełkowski et al. 1999; Golińska et al. 2002). No correlation was found between RAPD groupings and pathogenicity to wheat seedlings (Golińska et al. 2002). On a larger geographic scale, RAPD analysis of *F. avenaceum* isolates from Germany, France, and the U.K. revealed 100% genotypic diversity and that isolates did not cluster according to country of origin (Turner et al. 1998). *Fusarium avenaceum* populations have also been shown to be highly diverse on small spatial scales. Hybidization of the M13 probe to digested genomic DNA from 37 *F. avenaceum* isolates collected from 14 wheat ears from a single field plot revealed 14 different hybridization profiles (Nicholson et al. 1993). Outside of Europe, genetic analysis of *F. avenaceum* has been less common. Restriction fragment length polymorphism (RFLP) analysis of Australian *Fusarium* isolates was able to resolve each *F. avenaceum* isolate included in the study (Benyon et al. 2000). In North America, analysis of *F. avenaceum* has been limited to the PCR-RFLP analysis of six isolates, three from British Colombia and three from Idaho (Donaldson et al. 1995) and the multigene sequencing analysis of a worldwide *F. avenaceum* collection dominated by American isolates from lisianthus (Nalim 2004). Donaldson et al. (1995) was able to distinguish the three Canadian isolates from the American isolates. Nalim (2004) found no relation between plant host or geographic origin of *F. avenaceum* and genetic relatedness.

It is unclear why there is a lack of correlation between genetic relatedness and the geographic origin of *F. avenaceum*. Although traditionally considered a soil-borne pathogen, it can also be seed borne on lupin and other crops (Kollmorgen 1974; Nowicki 1995). Dispersal of the species on contaminated seed, whether of lupin or other species, may help to distribute genotypes throughout Alberta. Other long range dispersion mechanisms such as insect vectors (El-Hamalawi and Stanghellini 2005) and wind (Martin 1988) could explain the presence of closely related genotypes in distinct geographic areas, although these mechanisms have not been proven to occur in field situations.

Both mating type idiomorphs were found to occur in *F. avenaceum* and both mating types occurred at each location, except Penhold (Fig. 3-1). The presence of both mating types within a population is a requirement for sexual reproduction within a heterothallic species (Nelson 1996). The occurrence of mating types of *F. avenaceum* within natural populations has not been studied previously, although both mating types are known to exist within Europe (Kerényi et al. 2004). With both mating types of *F. avenaceum* found at the same locations, previous reports of naturally occurring teleomorphs in the species (Cook 1967; Booth and Spooner 1984) and active transcription of the *MAT* genes (Kerényi et al. 2004), it seems possible that sexual reproduction is occurring within the species.

The I_A tests suggest that some amount of recombination occurs within *F. avenaceum* (Table 3-3). Group I *F. avenaceum* isolates appear to be randomly mating, both locally and as an Alberta population. The lower *P* values found for the combined Group I isolates, compared to the individual locations, indicates that recombination is occurring mainly at a local scale. The data also suggest that the Group II isolates found at Barrhead and CDCN are recombining, but that the isolates recovered from Ellerslie are clonal. Similarly to Group I, recombination occurs locally, as the loci of the combined Group II isolates significantly differed from what is expected under linkage equilibrium. The results of the tests indicate that both Group I and Group II isolates are recombining, but most likely not with each other; the drop in *P* values when Group I and II isolates are combined indicates that they are not recombining with each other. The results from CDCN and Barrhead suggest that recombination has occurred within the groups, but infrequently between them. The I_A tests indicate that recombination occurs within *F. avenaceum*, but it does not clearly indicate what type of recombination occurs, its frequency, or when it occurred. Parasexuality, if it does occur in *F. avenaceum*, could produce results similar to sexual recombination (Taylor et al. 1999). Parasexuality has not been shown to be an important factor in fungal diversity *in vivo* for any species. In the populations that did not appear to be randomly mating, it is possible that sexual recombination is occurring, but in a nonrandom fashion, such as between close relatives.

It cannot be inferred if recombination is an ongoing process or if the patterns observed resulted from historical recombination.

Recombination within populations tends to have two major effects on fungal population structure: (1) relatively high levels of genotypic diversity and (2) random association between alleles at different loci (linkage equilibrium), which distinguish most sexual populations from asexual populations (Milgroom 1996). The combination of high genotypic diversity and populations within linkage equilibrium indicates that *F. avenaceum* is undergoing recombination. The presence of both mating types provides support for the occurrence of sexual recombination, as it could not occur without them. In *F. avenaceum* Group I, the mating type ratio is nearly 1:1, which is to be expected in a sexually reproducing population. There was clustering of mating types within Group I that is not easily explained. It could result from some of the markers used being linked to mating type, or from the asexual reproduction of isolates of the same mating type that were then distributed across Alberta. If sexual recombination is occurring, it could have a profound impact on attempts to control diseases caused by *F. avenaceum*. In fungal plant pathogens, the greatest importance of recombination is usually with respect to the development of new pathotypes that can overcome resistant varieties and fungicide resistance (Milgroom 1996). Sexual reproduction within *F. avenaceum* could also alter the epidemiology of the disease. With sexual reproduction comes the production of ascospores, which may have different dispersion patterns than hyphae or conidia. *Fusarium graminearum* ascospores are windborne and capable of traveling further distances than macroconidia, allowing disease to spread over long distances (Francl et al. 1999; Fernando et al. 2000; De Luna et al. 2002, Maldonaldo-Ramirez et al. 2005). The airborne ascospores of *F. graminearum*'s to become appear to have facilitated the creation of a large interbreeding population of the pathogen within North America (Schmale et al. 2006).

Sexual reproduction in *F. avenaceum* has been suggested before because of its high genotypic diversity (De Nijs et al. 1997), which is similar or greater than that of other *Fusarium* species which are known or suspected of having a sexual cycle. *Fusarium graminearum* reproduces sexually and *F. culmorum* is suspected of having a cryptic sexual cycle (Mishra et al. 2003; Tóth et al. 2004), based on population genetic analysis, yet the genetic diversity of these two species is lower than that of *F. avenaceum* (De Nijs et al. 1997; Chełkowski et al. 1999). Balancing selection is an alternative explanation that could be partially responsible for the high genotypic diversity found within *F. avenaceum*. The ability of the species to utilize both saprophytic and parasitic lifecycles and a wide host range could result in selection for saprophytic and parasitic traits causing the preservation of high levels of genotypic diversity, as has been suggested for other *Fusarium* spp. (Miedaner et al. 2008).

The division of the *F. avenaceum* isolates into two genetically distinct groups was not reflected in the morphology or pathogenicity of the isolates within the groups. The separation of *F. avenaceum* into multiple groups has been reported previously. Most Finnish *F. avenaceum* isolates have been divided into two groups by RAPD and UP-PCR analysis (Yli-Mattila et al. 1996; Yli-Mattila et al. 1997) and the division is supported by β-tubulin sequences. It is not known if the two groups reported here are related to the two main Finnish groups. It is also possible that one group represents *F. avenaceum* and the other *F. arthrosporioides*, although the two species appear to form a species complex (Yli-Mattila et al. 2002; Yli-Mattila et al. 2004) and not two separate monophyletic species. Species-specific primers typically used to identify *F. avenaceum* also amplify sequences from *F. arthrosporioides* and other closely related species (Turner et al. 1998; Yli-Mattila et al. 2004), and as such few researchers distinguish between the two species. Primers specific to isolates considered to be *F. arthrosporioides* have only recently been developed (Yli-Mattila et al. 2004).

Table 3-1. Primers used for the characterization of monoconidial *Fusarium* isolates collected from lupin in central Alberta and number of DNA bands analyzed.

Primer	Sequence	Number of DNA bands analyzed
Random		
UBC77[a]	GAGCACCAGG	11
P1[b]	AGGAGGACCC	12
Microsatellite[c]		
(GACA)$_4$	GACAGACAGACAGACA	1
(GTG)$_5$	GTGGTGGTGGTGGTG	9
(AAGC)$_4$	AAGCAAGCAAGCAAGC	8

[a] Random decamer primer reported by Chełkowski et al. (1999).

[b] Random decamer primer reported by Altomare et al. (1997).

[c] Microsatellite primers are reported by Balmas et al. (2005).

Table 3-2. Primers used for the molecular determination of the mating type of *F. avenaceum* isolates collected from lupin in central Alberta.

Primer[a]	Sequence	Mating type locus target
fusALPHAfor	CGCCCTCTKAAYGSCTTCATG	*Mat1-1*
fusALPHArev	GGARTARACYTTAGCAATYAGGGC	*Mat1-1*
fusHMGfor	CGACCTCCCAAYGCYTACAT	*Mat1-2*
fusHMGrev	TGGGCGGTACTGGTARTCRGG	*Mat1-2*

[a] Semidegenerate mating type primers developed by Kerényi et al. (2004).

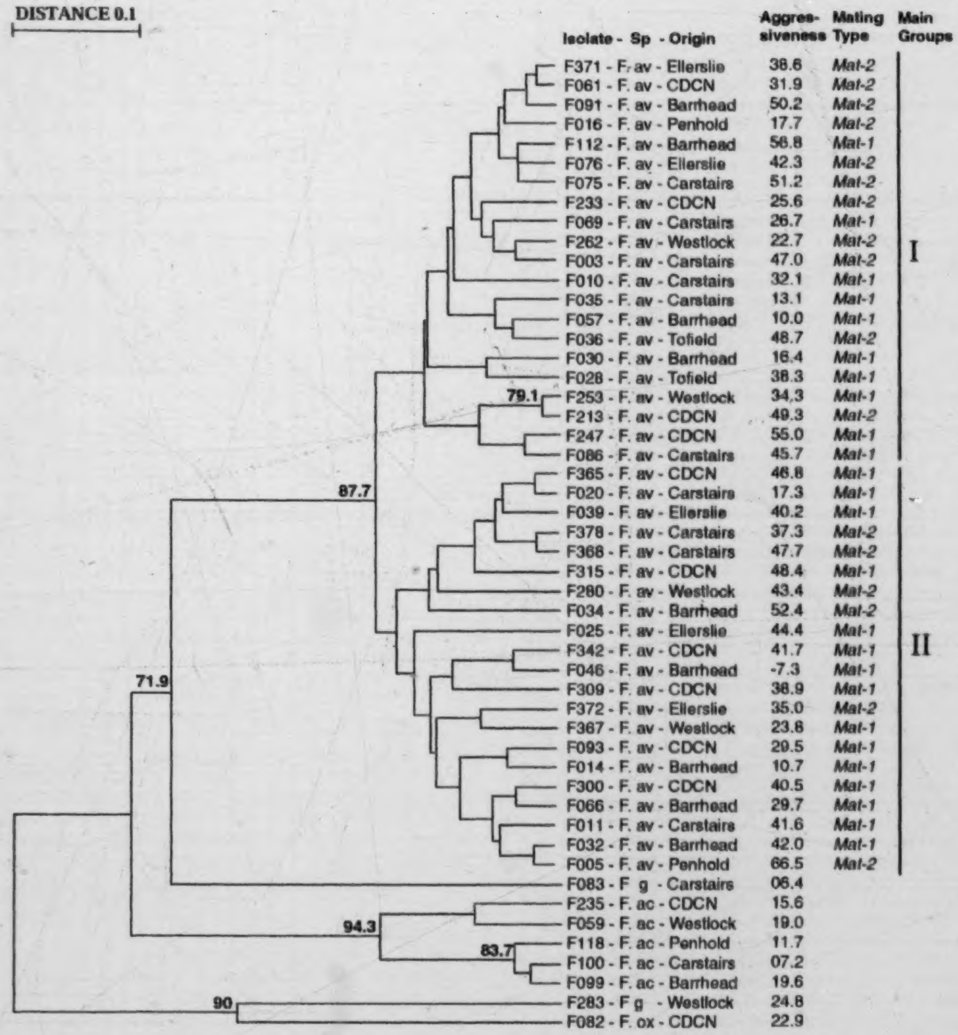
DISTANCE 0.1
Isolate - Sp - Origin
Aggres-siveness
Mating Type
Main Groups
F371 - F. av - Ellerslie 38.6 Mat-2
F061 - F. av - CDCN 31.9 Mat-2
F091 - F. av - Barrhead 50.2 Mat-2
F016 - F. av - Penhold 17.7 Mat-2
F112 - F. av - Barrhead 56.8 Mat-1
F076 - F. av - Ellerslie 42.3 Mat-2
F075 - F. av - Carstairs 51.2 Mat-2
F233 - F. av - CDCN 25.6 Mat-2
F069 - F. av - Carstairs 26.7 Mat-1
F262 - F. av - Westlock 22.7 Mat-2
F003 - F. av - Carstairs 47.0 Mat-2
F010 - F. av - Carstairs 32.1 Mat-1
F035 - F. av - Carstairs 13.1 Mat-1
F057 - F. av - Barrhead 10.0 Mat-1
F036 - F. av - Tofield 48.7 Mat-2
F030 - F. av - Barrhead 16.4 Mat-1
F028 - F. av - Tofield 38.3 Mat-1
79.1
F253 - F. av - Westlock 34.3 Mat-1
F213 - F. av - CDCN 49.3 Mat-2
F247 - F. av - CDCN 55.0 Mat-1
F086 - F. av - Carstairs 45.7 Mat-1
I
87.7
F365 - F. av - CDCN 46.8 Mat-1
F020 - F. av - Carstairs 17.3 Mat-1
F039 - F. av - Ellerslie 40.2 Mat-1
F378 - F. av - Carstairs 37.3 Mat-2
F368 - F. av - Carstairs 47.7 Mat-2
F315 - F. av - CDCN 48.4 Mat-1
F280 - F. av - Westlock 43.4 Mat-2
F034 - F. av - Barrhead 52.4 Mat-2
F025 - F. av - Ellerslie 44.4 Mat-1
F342 - F. av - CDCN 41.7 Mat-1
F046 - F. av - Barrhead -7.3 Mat-1
F309 - F. av - CDCN 38.9 Mat-1
F372 - F. av - Ellerslie 35.0 Mat-2
F367 - F. av - Westlock 23.8 Mat-1
F093 - F. av - CDCN 29.5 Mat-1
F014 - F. av - Barrhead 10.7 Mat-1
F300 - F. av - CDCN 40.5 Mat-1
F066 - F. av - Barrhead 29.7 Mat-1
F011 - F. av - Carstairs 41.6 Mat-1
F032 - F. av - Barrhead 42.0 Mat-1
F005 - F. av - Penhold 66.5 Mat-2
II
71.9
F083 - F g - Carstairs 06.4
94.3
F235 - F. ac - CDCN 15.6
F059 - F. ac - Westlock 19.0
83.7
F118 - F. ac - Penhold 11.7
F100 - F. ac - Carstairs 07.2
F099 - F. ac - Barrhead 19.6
90
F283 - F g - Westlock 24.8
F082 - F. ox - CDCN 22.9

Figure 3.1 – UPGMA dendrogram of genetic similarities between *Fusarium* spp. recovered from lupins in central Alberta, developed using Nei and Li's (1979) similarity coefficient. Bootstrap values (%) higher than 70 are shown at nodes. Species are *F. avenaceum* (F. av), *F. acuminatum* (F. ac), *F. oxysporum* (F. ox), and Fusarium section *Gibbosum* (F g). Aggressiveness is the percentage reduction in shoot weight compared to the control. Mating types and the main groups of *F. avenaceum* are shown.

Table 3-3. Tests for linkage equilibrium amongst *F. avenaceum* isolates collected from lupin in central Alberta.

	Group I				Group II				Group I and II			
Location	N	I_A	I^S_A	p	N	I_A	I^S_A	p	N	I_A	I^S_A	p
Barrhead	4	-0.017	-0.0017	0.702	5	0.766	0.0639	0.131	9	0.671	0.042	0.009
Carstairs	6	0.306	0.0236	0.286	2	-	-	-	8	0.482	0.0284	0.114
CDCN	4	0.263	0.0329	0.539	6	-0.052	-0.004	0.489	10	0.573	0.0286	0.042
Ellerslie	2	-	-	-	5	4	0.3333	0.001	7	1.70	0.1002	0.003
Penhold	1	-	-	-	1	-	-	-	2	-	-	-
Tofield	2	-	-	-	0	-	-	-	2	-	-	-
Westlock	2	-	-	-	2	-	-	-	4	1.205	0.0753	0.076
All locations	21	0.150	0.0083	0.152	21	0.301	0.0146	0.039	42	0.483	0.0179	<0.001

N = number of isolates, I_A = index of association, I^S_A = standardized index of association

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4. Characterization of the pathogenicity of *Fusarium avenaceum*

4.1 Introduction

Fusarium avenaceum (Fr.) Sacc. (teleomorph: *Gibberella avenacea* Cook), is a cosmopolitan soil-borne saprophyte and pathogen frequently associated with cereals and legumes, as well as numerous other hosts (Booth 1971). The species has long been recognized as causing seedling blight and root rot of *Lupinus angustifolius* L. (Wollenwebe and Reinking 1935; Weimer 1944). During experimental field trials evaluating *L. angustifolius* as a potential crop in central Alberta, Fusarium root rot was found to be the most common disease (Chang et al. 2005, 2006b) and *F. avenaceum* was the most prevalent and pathogenic *Fusarium* species isolated from lupins (Chapter 2).

Seedling blight and root rot caused by *F. avenaceum* have been reported previously on other legume species throughout Alberta (Cormack 1937; Hwang et al. 1994; Hwang et al. 2006). However, the role of *F. avenaceum* in root rot of lupin in Alberta and North America has not been assessed, but merits attention given the recent Albertan attempts to develop lupin as a crop.

Studies were undertaken to examine: 1) the effect of different concentrations of *F. avenaceum* on root rot and nodulation of lupin; 2) the effect of artificial seed contamination on seedling establishment and seed yield; 3) the combined effect of *F. avenaceum* and other commonly isolated *Fusarium* spp. on root rot of lupin; 4) the pathogenicity of *F. avenaceum* from the two previously identified UPGMA groups towards different lupin genotypes; and 5) the host range of *F. avenaceum* in crops that maybe grown in rotation with lupin in western Canada.

4.2 Materials and methods

4.2.1 Influence of *F. avenaceum* inoculum density on lupin root rot and nodulation

Inoculum of two *F. avenaceum* isolates (isolates 213 and 371) was produced in cornmeal-sand medium (CMS) (225 g sand (<1mm diameter), 25 g cornmeal (Unico Inc, Concord, ON), 50 mL distilled water) in 500 mL Erlenmeyer flasks. The CMS mixture was autoclaved for 90 min twice on subsequent days and sprinkled on PDA to check for contamination. *Fusarium* cultures were transferred from SNA to PDA and incubated at room temperature (RT) with a 12 hr photoperiod. Four 9-mm agar plugs of *F. avenaceum* from the growing edge of five-day-old colonies were used to inoculate the flasks. Control CMS was inoculated with sterile PDA pieces. CMS medium was then incubated for two weeks at RT and air dried in sterilized paper bags. Lumps in the dried CMS were ground and the numbers of colony forming units (CFU) were quantified through serial dilution on PCNB agar. CMS inoculum was stored in autoclaved plastic bags at 4°C until use.

Peat-based *Bradyrhizobium* sp. (*Lupinus*) inoculant (Novozymes Biologicals, Saskatoon, SK) was quantified by serial dilution onto Yeast-Mannitol Agar with Congo Red (YMA-CR) and tested for purity on Yeast-Mannitol Agar with Bromothymol Blue (YMA-BTB) and Peptone Glucose Agar with Bromcresol Purple (PGA-BCP) (Somasegaran and Hoben 1994).

Seeds of the lupin cultivars Arabella and Rose were surface sterilized in 1% (v/v) bleach for five minutes and rinsed five times with distilled water (Robinson et al. 2000).

Treatments consisting of differing densities of *F. avenaceum* were prepared by mixing colonized CMS with autoclaved potting mix (ProMix BX , Premier Horticulture, Dorval, QC) to produce inoculum densities of 5×10^3, 1×10^4, 2×10^4, 4×10^4, and 8×10^4 CFU g^{-1} of potting mix. The control treatment consisted of sterile CMS mixed with potting mix at the same rate as the 2×10^4 CFU g^{-1} treatment. The potting mix was used to fill small plastic cups (450 mL tuffcups, Georgia-Pacific, Dixie Business, Norwalk, CT) and tamped down. Eight cups were planted with Arabella and Rose, ten seeds per cultivar, per treatment. The inoculant was mixed with distilled water and 0.5 mL was pipetted over each seed, providing 3.16×10^6 cells per seed. The seeds were then covered with 1.5 cm of Promix. The experiment was arranged in a randomized block design. Emergence was recorded at two weeks, and at four weeks the plants were removed from their pots, their roots washed in tap water and the number of nodules per root and root rot severity were recorded. Root rot severity was recorded on a 0-5 scale modified from Hwang et al. (1994), where: 0 = healthy; 0.5 = tiny lesions on 0-12.5% of tap root; 1 = lesions on 12.5-25% side of tap root; 2 = lesions on 25– 49% side of tap root; 3 = brown lesions on 50–74% side of tap root and tap root constricted; 4 = tap root extensively girdled and brown lesions on >75% side of tap root, limited lateral roots present; 5 = plant dead. The individual ratings were converted to a mean root rot value by taking the quotient of the sum of the individual root rot ratings and the number of plants assessed in the same plot. The shoots were severed from the roots, dried and the dry weight of both the shoots and roots recorded. The experiment was repeated once.

4.2.2 Yield loss model

Experimental field plots were established in 2006 at Lacombe, AB, on a black chernozemic soil with a clay loam texture, and identical plots were established on May 24, 2007 at Edmonton, AB, on a black sandy loam chernozemic soil. The trials were laid out in a randomized complete block design with four replications. Treatments consisted of healthy lupin seed of cv. Arabella and lupin seed of cv. Arabella artificially inoculated with *F. avenaceum* at seven treatment rates: 0% (control), 5%, 10%, 25%, 50%, 75%, and 100% inoculated seed. Inoculated seed had been prepared at Alberta Agriculture and Rural Development, Lacombe, AB. Each subplot consisted of four rows, 6 m in length with 25 cm inter-row spacing. Seeds were planted about 5 cm deep at a rate of 50 seeds m^{-1}. Plots were spaced 1.2 m apart with 2 m between blocks. Seeds were sown with approximately 2.5 mL of the same Bradyrhizobium inoculant (Novozymes Biologicals, Saskatoon, SK) used in 4.2.1. Seedling establishment was counted at 27 days after seeding and seed yield was recorded for each plot.

4.2.3 Coinoculation of lupin with *F. avenaceum* and other *Fusarium* spp.

4.2.3.1 Greenhouse assay

Inoculum of *F. avenaceum* (isolate 247) was produced on CMS as in 4.2.1. Liquid inocula of the other *Fusarium* spp. used were produced in 250 mL Erlenmeyer flasks containing 150 mL of sterile Czapek-Dox broth (BD/Difco, Sparks, MD). The broth was inoculated with two 25 mm^2 pieces of agar colonized by *F. oxysporum* (isolates 145 and 351), *F. solani* (246), and *F. acuminatum* (263), with 2 flasks inoculated per isolate. Cultures were incubated at RT under the lighting conditions described above with continuous agitation at 150 rpm on an orbital shaker (Lab-Line Instruments Inc, Melrose Park, IL). . After 7 days, *F. oxysporum* and *F. solani* cultures were filtered through two layers of cheesecloth (EHI Inc, Niagara Falls, ON), centrifuged in 50 mL tubes for 3 minutes, then resuspended in sdH_20 and adjusted to 5×10^5 conidia mL^{-1}. The concentration of *F. acuminatum* inoculum was adjusted to 2.5×10^5 conidia and hyphal fragments mL^{-1}.

Treatments consisted of inoculations with each liquid *Fusarium* inoculum alone or in combination with *F. avenaceum* and a non-inoculated control. The experiment was set up in a completely randomized design (CRD). *Fusarium avenaceum*-colonized CMS was mixed with Promix BX potting mix (previously autoclaved for 90 minutes twice) for 30 minutes in a cement mixer (Scepter Corp., Scarborough, ON) to provide a inoculum density of 2×10^4 CFU g^{-1}. Sterile CMS was mixed with potting mix at the same rate to provide the control. *Fusarium avenaceum*-infested and noninfested potting mix were added to separate small plastic cups (450 mL tuffcups, Georgia-Pacific, Dixie Business, Norwalk, CT). Seeds of *L. angustifolius* cv. Arabella were surface-sterilized according to the method of Robinson et al. (2000) and ten seeds were added to each pot. Five mL of liquid inoculum was pipetted over all the seeds in each cup with one treatment per cup. The control consisted of noninfested potting mix with sterile distilled water pipetted over the seeds. There were eight replicate pots for each treatment combination. The pots were placed in a greenhouse with natural lighting and watered as required for three weeks. Emergence was recorded at two weeks, and at three weeks the plants were removed from their pots, their roots washed in tap water and the disease incidence and root rot recorded as described above. The shoots were severed from the roots, air dried and the dry weight measured. The experiment was repeated once. Due to higher than expected mortality in treatments incorporating *F. avenaceum*, the entire experiment was repeated two more times with the *F. avenaceum* concentration decreased to 5×10^3 CFU g^{-1}. *Fusarium acuminatum* was not included in these additional repetitions.

Reisolations were preformed on the roots according to the method of Hwang et al. (1994). Colonies were identified by colony and microscopic morphology on PDA and CLA, respectively.

4.2.3.2 Plate confrontation assay

The possible antagonism of *Fusarium* isolates to pathogenic *F. avenaceum* was tested *in vitro* in a dual culture assay. Plugs (4 mm diameter) were taken from the edge of colonies growing on SNA. Plugs of *F. avenaceum* and other *Fusarium* spp. were placed 5 cm apart on PDA plates. *Fusarium avenaceum* paired with sterile SNA plugs and *F. avenaceum* paired with *F. avenaceum* in dual culture served as controls. The experiment was set up in a CRD with four replicates per treatment. The plates were incubated at RT in the dark. After six days, two parameters were recoded: 1) the width of the zone of inhibition (ZI) and 2) the percentage inhibition of radial growth $[100 \times (r_1 - r_2)/r_1]$ (Fig 4-1). Plates were also measured at 12 days due to the slow growth of *F. acuminatum*. The experiment was repeated once.

4.2.4 Pathogenicity of *F. avenaceum* isolates on different lupin genotypes

In order to determine if the pathogenicity of *F. avenaceum* isolates varied on different lupin genotypes and if *F. avenaceum* isolates from the two UPGMA groups differed in pathogenicity, a greenhouse experiment was performed.

Fusarium avenaceum isolates (Table 4-1) 028, 036, 213, 233, 247, 262 (UPGMA group 1) and 011, 014, 025, 365, 372, 378 (UPGMA group 2), and *F. acuminatum* isolates (100 and 235) were used. Fungal inoculum was produced in CMS and the inoculum was then adjusted to a concentration of 1×10^5 CFU g^{-1} with sterilized sand.

Five different lupin genotypes were selected as hosts: cultivars Arabella and Rose and lines E8, G851, and MLU-320. Seeds were surface-sterilized according to Robinson et al. (2000).

Thirty-eight cell planting trays (ITML Horticultural Products Inc, Brantford, ON) were filled with autoclaved Promix BX potting mix, previously autoclaved for 90 minutes. The potting mix was tamped down with a 45 mm diameter tamp and five seeds of the same genotype were added to each cell. At seeding, 2.8 g of the inoculum was spread overtop of the seeds, providing 2.8×10^5 CFU of inoculum (1.76×10^4 CFU cm^{-2}). Sterilized sand served as a control. The treatments were applied in a randomized complete block design (RCBD) with eight replicates, determined by the program EDGAR (www.edgarweb.org.uk). Trays were maintained in a greenhouse with natural lighting. Emergence was recorded at two weeks. Disease incidence, root rot severity, and shoot dry weight were determined as in 4.2.1 at three weeks. The experiment was repeated once.

4.2.5 *Fusarium avenaceum* host range

Seed of alfalfa (*Medicago sativa* L.) cv. Anchor, barley (*Hordeum vulgare* L.) cvs. Harrington and Vivar, bean (*Phaseolus vulgaris* L.) cv. CDC Pintium, birdsfoot trefoil (*Lotus corniculatus* L.) cv. Leo, canola cvs. Invigor 52 (*Brassica napus* L.) and Hysyn 10 (*Brassica rapa* L.), chickpea (*Cicer arietinum* L.) cvs. Chico and Myles, yellow sweet clover (*Melilotus officinalis* L.) cv. Yellow Blossom, fababean (*Vicia faba* L.) cv. Snowbird, flax (*Linum usitatissimum* L.) cv. Solin, lentil (*Lens culinaris* Medik.) cvs. black, green, and red, lupin (*L. angustifolius*) cv. Arabella, oats (*Avena sativa* L.) cv. Mustang (hulled) and cv. LAO 790 (hulless), pea (*Pisum sativum* L.) cv. Cutlass, rye (*Secale cereale* L.) cv. AC Rifle, soybean (*Glycine max* (L.) Merr.) cv. Gaillard, triticale (X *triticosecale* Wittmack) cv. Pronghorn (spring) and cv. Bobcat (winter), and wheat (*Triticum aestivum* L.) cv. AC Vista (spring) and cv. AC Radiant (winter) were surface-sterilized and planted. Ten seeds were planted per pot with 10 replications (pots) per treatment and inoculum of *F. avenaceum* was added onto the soil surface. Noninfested wheat:sand (4:1 v/v) inoculum was used as the control. Emergence was recorded at ten days after planting, plant height, root length, plant fresh and dry weights, and root rot severity (0-9 scale) were recorded at six weeks.

4.2.6 Statistical analysis

All analyses were conducted using SAS software (version 9.1.3, SAS Institute Inc., Cary, NC) except regression analysis, which was conducted using the software SigmaPlot (version 10.0, SPSS Inc., Richmond, CA).

4.2.6.1 Inoculum density and nodulation

The data were subjected to analysis of variance using PROC MIXED. Data from the two different cultivars were combined, as there was no significant difference between the cultivars for any of the response variables measured (P>0.05). Linear and nonlinear regression was used to determine the relation between inoculum density and emergence, root rot, nodulation, and shoot and root dry weight. The relationship between nodulation and inoculum density, root rot, and root weight was determined by correlation analysis using PROC CORR.

4.2.6.2 Yield loss model

Data from the yield loss model were analyzed using the mixed procedure with seed inoculation as the fixed effect and blocks and experiment effects as random effects. Means were compared using Tukey-Kramer pairwise comparisons and letter groupings generated using the macro of Saxton (1998).

4.2.6.3 Coinoculation of lupin with *F. avenaceum* and other *Fusarium* spp.

4.2.6.3.1 Greenhouse assay

Data on shoot dry masses, emergence, root rot severity, and disease incidence were analyzed using analysis of variance conducted using PROC MIXED of SAS. The different inoculations were treated as fixed effects and the replications of the trials as random blocks. Differences between least significant means were tested using Tukey-Kramer pairwise comparisons and letter groupings generated using the macro of Saxton (1998). The experiments involving the high and low concentrations of *F. avenaceum* were analyzed separately.

4.2.6.3.2 Plate confrontation assay

The inhibition of radial growth (RGI) of *F. avenaceum* and the zone of inhibition (Zi) were analyzed using PROC MIXED of SAS. The pairings of isolates in dual culture was treated as a fixed effect and the replications of the experiment as a random effect. Differences between least significant means were tested using Tukey-Kramer pairwise comparisons and letter groupings generated using the macro of Saxton (1998).

4.2.6.4 Pathogenicity of *F. avenaceum* isolates on different lupin genotypes

Analysis was carried out in SAS using the PROC MIXED program. The replicates of the experiments and blocks were treated as random effects with experimental blocks nested within replicates. Lupin genotype and *Fusarium* isolate were treated as fixed effects. Root rot severity was transformed using a $\log_{10}(x+1)$ transformation and disease incidence data were arcsine transformed to improve normality. Multiple comparisons were performed using Tukey-Kramer pairwise comparisons and letter groupings were generated using the macro of Saxton (1998). Single degree of freedom contrasts were preformed to compare *F. avenaceum* isolates from each UPGMA group for all genotypes combined and for each individual genotype separately. Where applicable, detransformed data are shown.

4.2.6.5 *Fusarium avenaceum* host range

Inoculated and noninoculated treatments were compared using PROC GLM. Multiple comparisons were conducted using the step-down Bonferroni adjustment in PROC MULTTEST due to the large number of paired comparisons.

4.3 Results

4.3.1 Influence of *F. avenaceum* inoculum density on lupin root rot and nodulation

Minor root rot occurred rarely within the control treatments. In contrast, the majority of plants in the treatments incorporating *F. avenaceum* suffered root rot. For both *F. avenaceum* isolates emergence decreased with increasing inoculum density (Fig. 4-2 A) By 4×10^4 CFU g^{-1} emergence had been reduced by 50% by isolate 371. For isolate 213 emergence continued to decrease until the highest inoculum concentration. Disease severity increased exponentially with increasing inoculum density of both isolates (Fig. 4-2 B). Isolate 371 caused more severe root rot than isolate 213, but for both isolates average disease severity reached a plateau when inoculum density was near 2×10^4 CFU g^{-1}. The number of nodules per root decreased exponentially with increasing inoculum density (Fig. 4-3 B). For isolates 371 an inoculum density of 4×10^4 CFU g^{-1} or higher almost completely eliminated nodulation. Significant correlations ($P<0.05$) were found between increasing inoculum density and decreasing shoot and root weight for isolate 371, but no model fit the data well ($r^2=0.24$ and 0.09). There was no strong relation between shoot or root weight and inoculum density for isolate 213 ($r^2=0.02$ and 0.002, respectively) (Fig. 4-4 D and E).

Correlation analysis demonstrated that nodulation was significantly correlated ($P \leq 0.001$) with inoculum density and root rot severity (Table 4-2).

4.3.2 Yield loss model

Inoculation of lupin with *F. avenaceum* reduced ($P<0.0001$) seedling establishment and ($P<0.0001$) seed yield (Table 4-3). All levels of *Fusarium* inoculation decreased seedling establishment and seed yield compared to the non-inoculated control. The difference between seedling emergence for the control and inoculated seeds was not significant until 25% of the seeds were coated with *Fusarium*. With respect to yield, the difference between control and inoculated seeds was not significant until seed contamination reached 75%.

4.3.3 Coinoculation of lupin with *F. avenaceum* and other *Fusarium* spp.

4.3.3.1 Greenhouse assay

Results of the greenhouse experiments showed that treatments incorporating *F. avenaceum* adversely affected lupin seedlings, as expected (Fig. 4-3, Fig. 4-4). Shoot weight and emergence were significantly decreased and root rot severity and disease incidence were significantly increased by treatments that included *F. avenaceum*. Shoot weight was significantly affected by treatments incorporating high concentrations of *F. avenaceum* ($P<0.001$) and low *F. avenaceum* concentrations ($P<0.0053$). In the experiments using the high *F. avenaceum* concentration, there were no significant differences in shoot weights among the treatments that included *F. avenaceum* and there were no significant differences among treatments that did not include *F. avenaceum* (Fig. 4-3A). In the experiment using a lower *F. avenaceum* concentration, inoculation with *F. avenaceum* alone or in combination significantly reduced shoot weight compared to the control except for co-inoculation with *F. oxysporum* 351 (Fig. 4-4A). In both experiments, however, the greatest reduction in shoot weight occurred when seeds were inoculated only with *F. avenaceum*. When a second *Fusarium* spp. was included, the reduction was not as severe. Root rot severity and disease incidence both significantly increased ($P<0.0001$) in both coinoculation experiments. Multiple comparisons showed that there were no significant differences amongst treatments including *F. avenaceum* or treatments excluding *F. avenaceum*. In both experiments, the most severe root rot occurred when only *F. avenaceum* was used to inoculate seeds (Fig. 4-3B; Fig. 4-4B). Only minor differences in disease incidence occurred in the experiments using a high *F. avenaceum* concentration. The treatments including *F. avenaceum* alone or with either of the two *F. oxysporum* isolates all had a disease incidence of 100%, while *F. avenaceum* co-inoculated with *F. solani* or *F. acuminatum* had disease incidences of 99% (Fig. 4-3C). The differences among treatments were greater in the subsequent experiment with a reduced *F. avenaceum* concentration (Fig. 4-4C). *Fusarium avenaceum* with *F. oxysporum* 145, *F. avenaceum* alone, and *F. avenaceum* with *F. oxysporum* 351 had disease incidences of 90%, 89%, and 88%, respectively, while coinoculation with *F. solani* resulted in a disease incidence of with 83%. Treatment combinations also had a significant effect on emergence in both experiments ($P<0.001$ and $P<0.0004$). The lowest emergence in both experiments occurred with the inoculation of *F. avenaceum* alone, and unlike all inoculations with *F. avenaceum* and another *Fusarium* isolate, it was the only treatment that was significantly different from all treatments that did not include *F. avenaceum* (Fig. 4-3D; Fig. 4-4D).

All *Fusarium* species could be reisolated from the roots of plants that they had inoculated, both when inoculated alone or in combination.

4.3.3.2 Plate confrontation assay

The *in vitro* experiments examining the potential antagonistic activity of *Fusarium* spp. towards *F. avenaceum* showed no *Fusarium* isolate significantly reduced radial growth more than *F. avenaceum* paired with itself in dual culture (Table 4-4). No stable zone of inhibition formed between *F. avenaceum* and any of the *Fusarium* isolates tested (Table 4-4) and with time, all colonies grew together. Due to the slow growth of *F. acuminatum*, an additional eight days was required to determine if there was a zone of inhibition.

4.3.4 Pathogenicity of *F. avenaceum* isolates on different lupin genotypes

Both *Fusarium* isolate and lupin genotype had a significant effect on all response variables measured, with the exception of the effect of genotype on shoot weight (Table

4-5). There were no significant interactions between the two factors. The genotype MLU-320 suffered from significantly greater root rot and decreased emergence than the other cultivars (Fig. 4-5). Despite this, the highest dry shoot weight occurred with this genotype. The opposite situation occurred with genotype E8, which suffered significantly less root rot than the other genotypes and had higher emergence than all other cultivars, but produced the least amount of above ground biomass (Fig. 4-5). Cultivars Arabella and Rose and line G851 were not significantly different for any of the response variables analyzed. The two *F. acuminatum* isolates included in the study and *F. avenaceum* 014 were not significantly pathogenic, based on any of the factors measured (Fig. 4-6). All other *F. avenaceum* isolates were capable of causing root rot significantly more severe ($P \leq 0.05$) than the control.

Inoculation with *F. avenaceum* from UPGMA Group I caused significantly more disease incidence and root rot for each line than did isolates from UPGMA Group II (Table 4-6). Emergence was consistently lower for all genotypes when inoculated with *F. avenaceum* from UPGMA Group II than UPGMA Group I, the difference was only significant for cv. Rose. There were no significant differences in shoot weight between lupins inoculated with the *F. avenaceum* from the different groups.

4.3.5 *Fusarium avenaceum* host range

Fusarium avenaceum was capable of causing significant reductions ($P \leq 0.05$) in the emergence of all hosts tested except bean and hulled oats (table 4-7). Plant height was significantly ($P \leq 0.05$) reduced for all cultivars except bean and soybean and root length was reduced for all cultivars except bean, soybean, and barley cv. Harrington. Inoculation decreased the fresh and dry weights of all cultivars, except beans that had no reduction in dry weight. All oilseed and legume crops, except beans, chickpea cv. Myles, peas, and soybeans suffered significant ($P \leq 0.05$) root rot when inoculated with *F. avenaceum*. No cereal cultivars suffered any root rot. For all legumes that suffered significant levels of rot, the damage was on average, more severe than that of lupin.

4.4 Discussion

4.4.1 Influence of *F. avenaceum* inoculum density on lupin root rot and nodulation

The increase in disease severity with increasing inoculum concentration is not surprising, and similar trends have been seen before in other legume crops (Hwang et al. 1994). The lack of any significant difference between Arabella and Rose is probably indicative of their origin from breeding programs that attempted to increase resistance to *F. avenaceum* (Kutpsov et al. 2006). The regressions indicate the relationships between inoculum density and root rot and nodulation were stronger with isolate 371 (r^2 of 0.82 and 0.72 for root rot and nodulation, respectively) than for isolate 213 (r^2 of 0.45 and 0.39 for root rot and nodulation, respectively) (Fig 4-2). These results may reflect the natural variation in pathogenicity between *F. avenaceum* isolates. Isolate 213 appeared to be less aggressive, producing less severe root rot than isolate (Fig. 4-2B). The decrease in nodulation was strongly negatively correlated with root rot severity, but not root weight, suggesting that the health of lupin roots is more important for the initiation and development of nodules than the size of the roots (Table 4-2). The decrease in nodulation would probably result in reduced grain yield and quality and a reduced ability to act as a green manure crop due to reduced nitrogen fixation.

4.4.2 Yield loss model

Artificial inoculation of lupin with *F. avenaceum* reduced stand establishment and decreased seed yield (Table 4-3). *Fusarium* spp. has not been implicated as a common or important seedborne disease of *L. angustifolius* within Alberta (Chang et al. 2005). *Fusarium avenaceum* has previously been shown to be seedborne in *L. angustifolius* (Nowicki 1995), but at lower levels than used here. *Fusarium avenaceum*, although capable of causing severe reductions in the yield of *L. angustifolius* when present at high levels, was not a significant problem at lower concentrations.

4.4.3 Coinoculation of lupin with *F. avenaceum* and other *Fusarium* spp.

As was previously found (Chapter 2), the inoculation of lupins with individual *Fusarium* species indicated that only *F. avenaceum* was capable of causing severe root rot or reducing seedling emergence (Figs. 4-3 and 4-4). *Fusarium avenaceum*, like all soil fungi, does not inhabit soil and the rhizosphere in isolation. Interactions between *Fusarium* species can alter the progression and severity of disease. Coinoculation of *F. avenaceum* with other *Fusarium* spp. has been found to increase, decrease or have no effect on disease severity. Wong et al. (1984) found that root rot of clover was more severe when pathogenic *F. avenaceum* and weakly pathogenic *F. oxysporum* were inoculated together, rather than when applied alone. Bateman (1997) found that root rot of white lupin was no more severe when pathogenic *F. avenaceum* and *F. solani* were simultaneously applied to lupin than when *F. avenaceum* was used alone. A reduction in root rot of chickpea caused by *F. avenaceum* has been found when the plants are inoculated with additional *Fusarium* species (Demirchi et al. 1999). The experiments in this study show that there was a statistically nonsignificant but consistent trend towards decreased disease severity and increased emergence and shoot weight when a second *Fusarium* species was applied in addition to *F. avenaceum* (Figs. 4-3 and 4-4).

No synergistic effects on pathogenicity were observed. The inability of the other *Fusarium* isolates to cause significant disease when inoculated alone probably reduced the chance of this occurring. It was not an impossibility however, since avirulent *F. solani* isolates have previously been shown to significantly increase the severity of other root diseases (Peters and Grau 2002). The reduction in disease severity of plants exposed to multiple fungal isolates or species has generally been attributed to several factors including: competition for colonization sites and nutrients, induction of plant resistance mechanisms, antibiosis and parasitism (Whipps 2001). There is evidence that *Fusarium* species are capable of using all of these methods to reduce the efficacy of plant pathogens. The majority of research is based on the study of non-pathogenic *F. oxysporum* strains. Competition for nutrients and colonization sites between non-pathogenic and pathogenic *F. oxysporum* have been implicated as the cause for the reduction in root rot and wilt caused by *F. oxysporum* (Mandeel and Baker 1991; Bolwerk et al. 2005; Olivain et al. 2006). Colonization of upper layers of root cells by nonpathogenic *F. oxysporum* can also cause induced systemic resistance (ISR) (Fuchs et al. 1997; Larkin and Fravel 1999). The successful reisolation of all *Fusarium* species inoculated onto roots indicates that all species involved in the study were capable of colonizing lupin roots. The growth of the isolates could have decreased nutrients available for *F. avenaceum* and the successful colonization of the roots may have partially excluded *F. avenaceum* while triggering lupin resistance mechanisms. *Fusarium oxysporum* has also been shown to exhibit antibiosis and act as a mycoparasite against non-Fusarium species (Benhamou et al. 2002; Rodríguez et al. 2006). However, the lack of a significant reduction in growth or zones of inhibition in the plate confrontation assay are suggestive that antibiosis did not occur in this study.

It is unclear if the results of the experiment would be applicable to all *F. avenaceum* isolates pathogenic towards lupin, as only one isolate of *F. avenaceum* was included. Maèkinaitë (2005) tested the *in vitro* reaction of ten *F. avenaceum* isolates against isolates of *F. oxysporum* and other *Fusarium* species and found that the reactions varied among isolates of the same species. Most reactions between *F. avenaceum* and *F. oxysporum* were classified as fungistatic, but mutual antagonism did occur in certain isolate pairings. Due to the use of only one *F. avenaceum* isolate, it is also not clear if the reduction in disease severity that occurred with two different *Fusarium* spp. would occur with different *F. avenaceum* isolates. Miedaner (2004) showed that the severity of FHB on rye caused by *F. culmorum* is decreased when multiple *F. culmorum* isolates were used as an inoculum, compared to single isolate inoculations.

The study indicates that the different *Fusarium* species were not part of a disease complex of lupins. Instead, *F. avenaceum* was the main pathogen and the other species were saprophytes. As the study was limited in duration, the results and early root rot symptoms may not be totally indicative of the actual situation occurring in fields later in the season.

4.4.4 Pathogenicity of *F. avenaceum* isolates on different lupin genotypes

The lack of any interaction between *Fusarium* isolate and lupin genotype was not unexpected, since *F. avenaceum* isolates are known to have a very wide host range and are not typically divided into host-specific pathotypes (Schneider 1958; Hwang et al. 1994).

The difference in pathogenicity between the group I and II *F. avenaceum* isolates may be attributable to the presence of one non-pathogenic *F. avenaceum* isolate in the study. If this isolate is removed from the contrast analysis, there is no longer a significant difference between root rot severity for any variety or cultivar, except Arabella. Group I isolates, however, still cause a significantly higher disease incidence and Group II causes a significant reduction in lupin emergence. The differences in pathogenicity between the two *F. avenaceum* groups found in this experiment are not what were found previously (Chapter 2). In the preliminary test, involving more isolates, the severity of root rot or reduction in emergence caused by Group I and Group II isolates were not significantly different.

Little work has been done to correlate the pathogenicity of *F. avenaceum* to the genetic structure of pathogen populations. Satyaparasad et al. (2000) found the pathogenicity of *F. avenaceum* from white lupin was not related to either the RAPD profiles of the isolates nor to the two RFLP groups the isolates were divided into.

The difference in the significance in root rot and emergence between the two groups could also be caused in part by the rating scale used. Because seedlings that did not emerge did not contribute to the root rot rating, isolates that caused post-emergence root rot and seedling blight would be recorded as causing more severe disease than isolates that caused pre-emergent damping off.

Fusarium acuminatum was found to be nonpathogenic to lupin in this study. Of the two isolates used, one had previously been found to cause minor root rot on lupin (Chapter 2). It is possible that the earlier preliminary result was wrong or that the isolate lost its virulence during the time it was in storage. It is possible that the lack of pathogenicity was due to elevated temperature, since the experiment was performed in the summer, while the preliminary pathogenicity tests were conducted in the winter. The growth and survival of *F. acuminatum* are known to be detrimentally affected by high temperatures (Sangalang et al. 1995a,b).

4.4.5 *Fusarium avenaceum* host range

The results support the known broad host range of *F. avenaceum*. Isolates of *F. avenaceum* from Alberta have been previously reported as pathogenic on all species found to be susceptible in this study (Cormack 1937; Calman et al. 1986; Hwang et al., 2000; Benard and Lange 2002; Wang et al. 2003; Chang et al. 2006; Hwang et al. 2006) with the notable exceptions of soybean, fababean and chickpea which have been found to be susceptible to *F. avenaceum* in other regions (Schneider 1958; Cother 1977; Sturz and Carter 1995).

The host range of *F. avenaceum* collected from lupin crops in central Alberta is comparable to that reported in European host range studies involving lupins and other legumes. The Danish Research Centre for Organic Food and Farming (DARCOF) (2005) found that *F. avenaceum* pathogenic to *L. angustifolius* was also pathogenic to fababean and pea, as was found here. In a large host range experiment, Schneider (1958) tested three different *F. avenaceum* isolates from *L. angustifolius* and seven isolates from *L. albus* and *L. luteus*, as well as 26 other isolates from carnation, cereal, and apple on legume and cereal crops. In agreement with the results here, isolates from *L. angustifolius* were pathogenic on lupin, faba bean, pea, alfalfa and clover, but caused only very minor disease symptoms on beans. *Fusarium avenaceum* from lupin was also pathogenic on wheat, rye, and barley. The isolates from the other crop plants produced similar results. Satyaparasad (2000) also found that *F. avenaceum* recovered from white lupin could cause root rot on wheat. The reduction in emergence of cereal crops without any root rot is in contrast to many other studies of *F. avenaceum*, where it is capable of causing severe root rot (Schneider 1958; Celetti et al. 1990).

Flax and canola, although highly susceptible to the *F. avenaceum* strain used in this experiment, do not usually suffer major problems associated with *F. avenaceum*. In Saskatchewan, *F. avenaceum* has been isolated from roots of canola and flax, but was not statistically associated with root discolouration (Fernandez 2007).

Due to the wide host range of *F. avenaceum* from lupin, as well as its ability to survive saprophytically, it is probable that *F. avenaceum* field population levels could remain stable throughout rotations involving lupins. Dry bean could be a preferred pulse crop in areas with high *F. avenaceum* levels, given its low susceptibility to this pathogen.

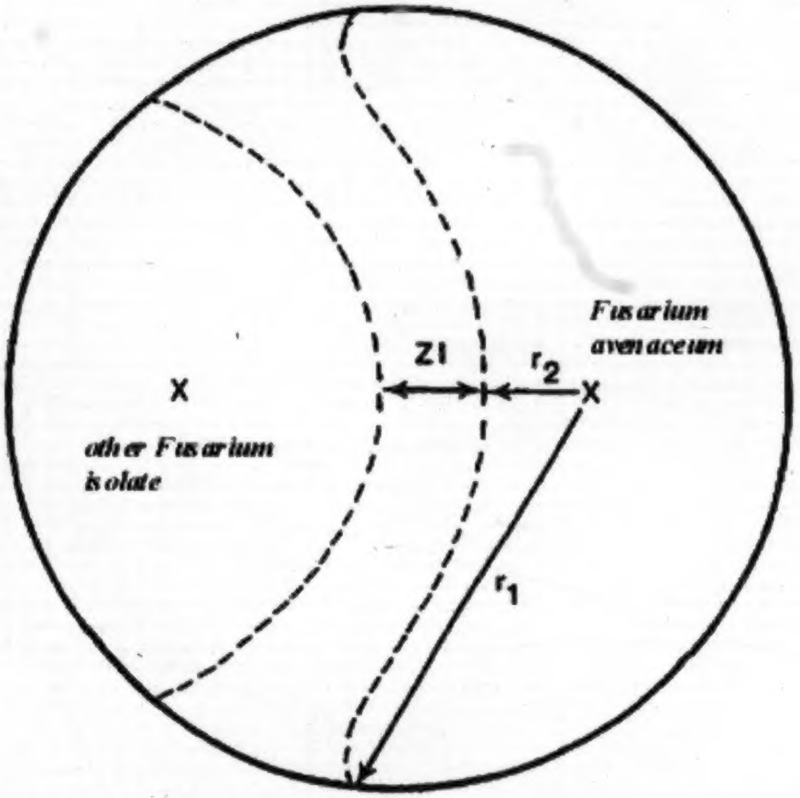
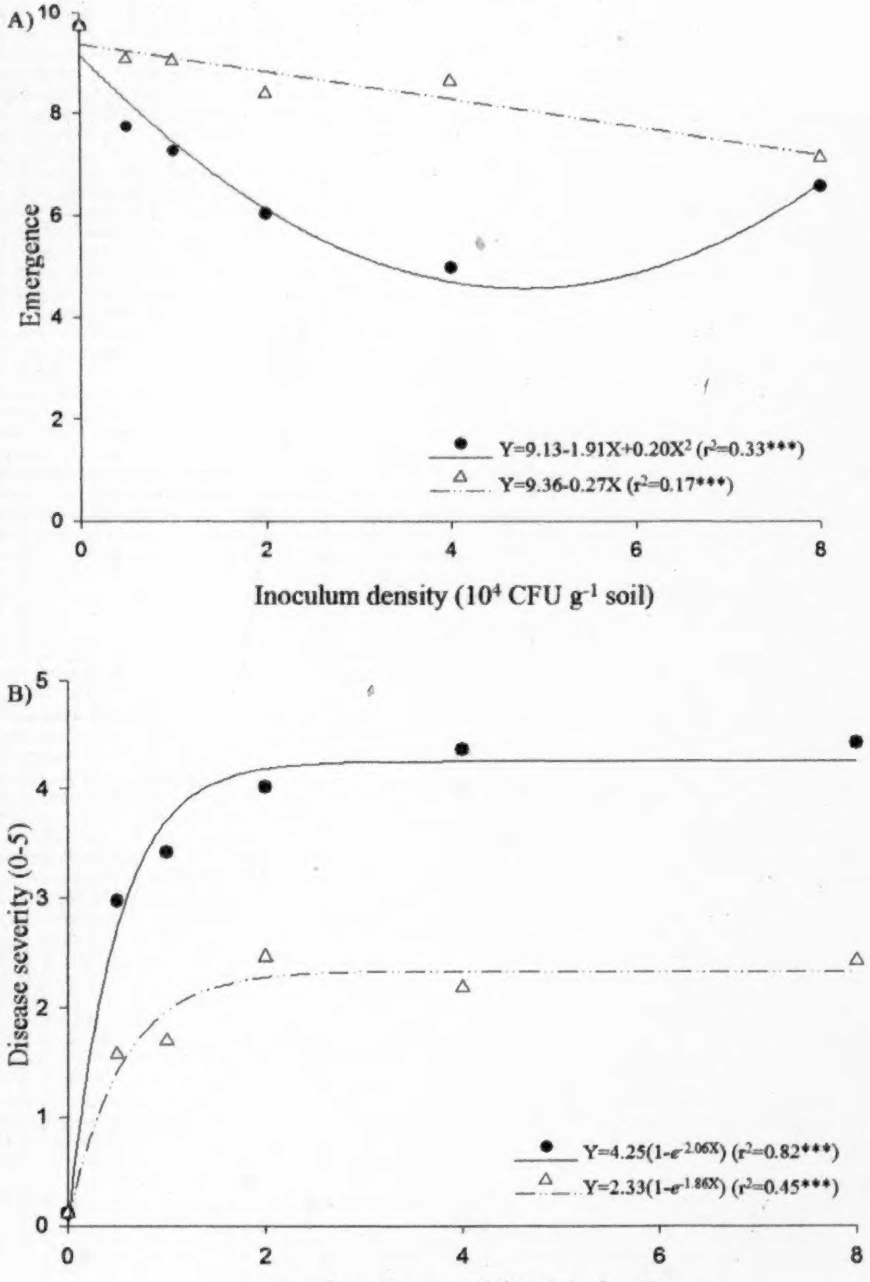

Fusarium avenaceum
ZI
r_2
X
X
other Fusarium isolate
r_1

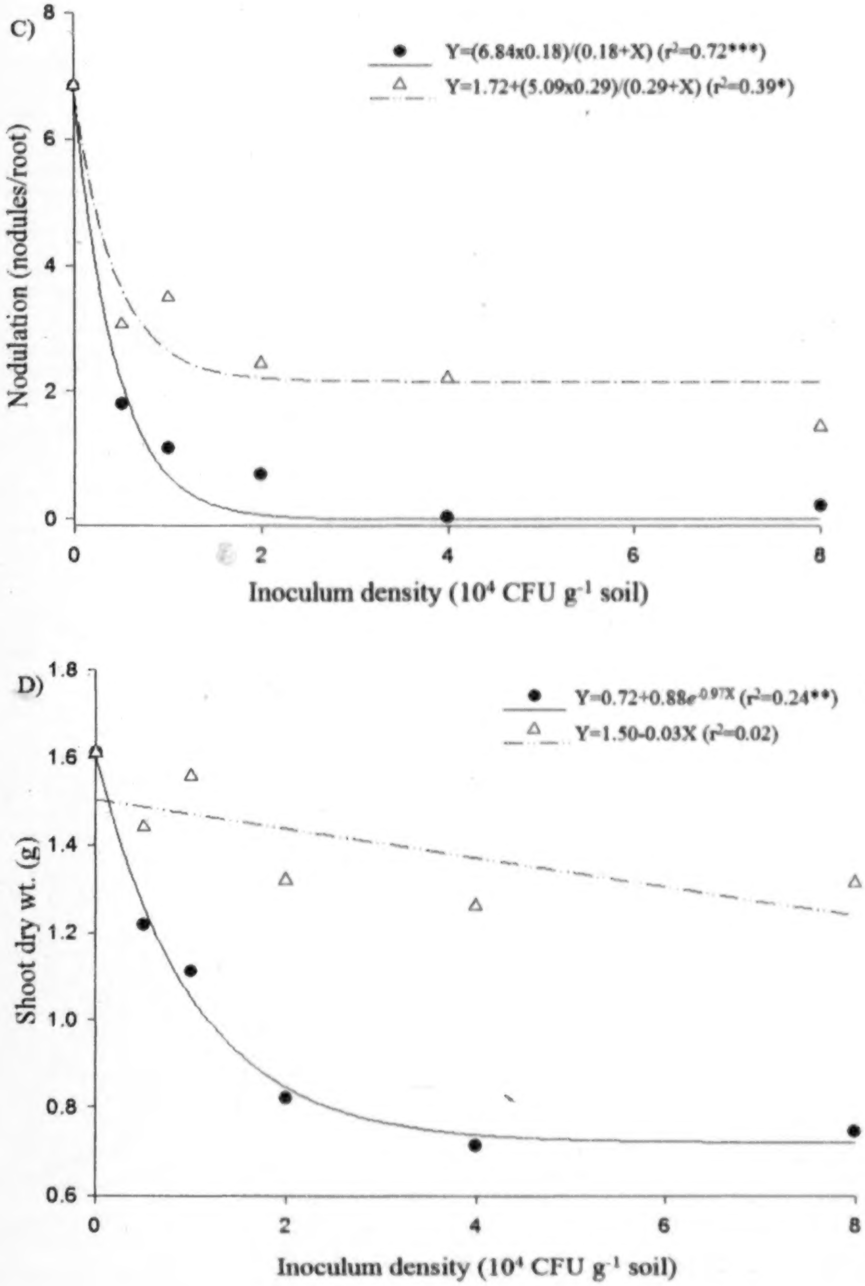
Figure 4-1. Diagram of the agar plate confrontation assay. Parameters are the percentage inhibition of radial growth (RGI) [$100x(r_1-r_2)/r_1$] and the width of the zone of inhibition (ZI). Adapted from Royse and Ries (1978).

Table 4-1. Isolates used for the comparison of the pathogenicity of *F. avenaceum* on different lupin genotypes.

Isolate Number	Species	Origin	UPGMA Group	Mating Type
028	*F. avenaceum*	Tofield	I	*Mat1-1*
036	*F. avenaceum*	Tofield	I	*Mat1-2*
213	*F. avenaceum*	CDCN	I	*Mat1-2*
233	*F. avenaceum*	CDCN	I	*Mat1-2*
247	*F. avenaceum*	CDCN	I	*Mat1-1*
262	*F. avenaceum*	Westlock	I	*Mat1-2*
011	*F. avenaceum*	Carstairs	II	*Mat1-1*
014	*F. avenaceum*	Barrhead	II	*Mat1-1*
025	*F. avenaceum*	Ellerslie	II	*Mat1-1*
365	*F. avenaceum*	CDCN	II	*Mat1-1*
372	*F. avenaceum*	Ellerslie	II	*Mat1-2*
378	*F. avenaceum*	Carstairs	II	*Mat1-2*
100	*F. acuminatum*	Carstairs	NA	Unknown
235	*F. acuminatum*	CDCN	NA	Unknown


A)
Emergence
Y=9.13-1.91X+0.20X² (r²=0.33***)
Y=9.36-0.27X (r²=0.17***)
Inoculum density (10⁴ CFU g⁻¹ soil)
0
2
4
6
8
10
B)
Disease severity (0-5)
Y=4.25(1-e^-2.06X) (r²=0.82***)
Y=2.33(1-e^-1.86X) (r²=0.45***)
Inoculum density (10⁴ CFU g⁻¹ soil)
0
1
2
3
4
5
6
8

(continued)


C)
Nodulation (nodules/root)
0
2
4
6
8
● Y=(6.84x0.18)/(0.18+X) (r^2=0.72***)
△ Y=1.72+(5.09x0.29)/(0.29+X) (r^2=0.39*)
0
2
4
6
8
Inoculum density (10^4 CFU g^{-1} soil)
D)
Shoot dry wt. (g)
0.6
0.8
1.0
1.2
1.4
1.6
1.8
● Y=0.72+0.88$e^{-0.97X}$ (r^2=0.24**)
△ Y=1.50-0.03X (r^2=0.02)
0
2
4
6
8
Inoculum density (10^4 CFU g^{-1} soil)

(continued)

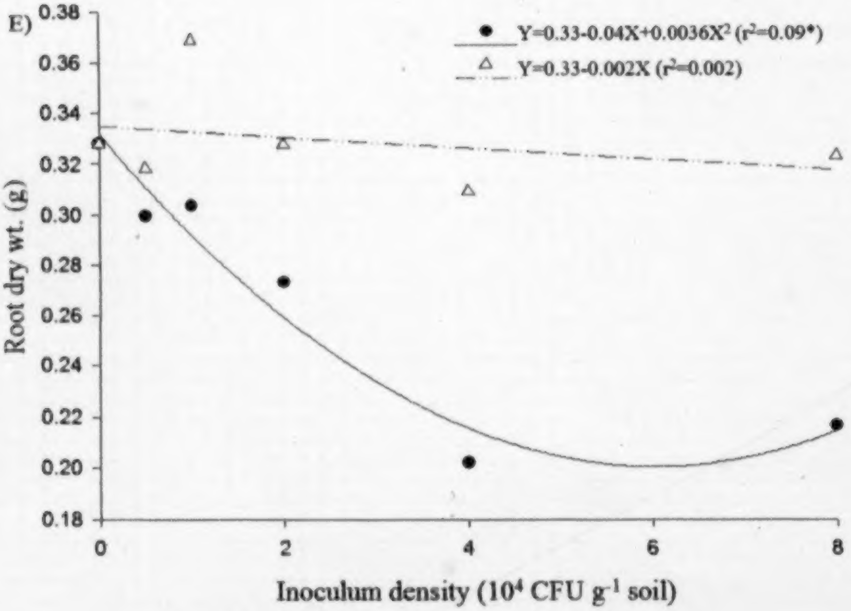


Figure 4-2. Relationship between inoculum density of *Fusarium avenaceum* and lupin A) emergence, B) root rot, C) nodulation, D) shoot dry weight, and E) root dry weight. Symbol ● represents *F. avenaceum* isolate 371 and △ *F. avenaceum* isolate 213. Each data point represents the mean of 32 replicates with ten plants per replicate. * indicates significance at $P=0.05$ ** indicates significance at $P=0.01$, *** indicates significance at $P=0.001$.

Table 4-2. Correlation of the number of nodules per root of lupin cv. Arabella or Rose with *F. avenaceum* inoculum density, root rot, shoot weight, and root weight isolates 213 and 371.

	F. avenaceum 213		*F. avenaceum* 371	
	Arabella	Rose	Arabella	Rose
	r[a]	r	r	r
Density	-0.54 ***	-0.53 ***	-0.67 ***	-0.79 ***
Root Rot[b]	-0.58 ***	-0.65 ***	-0.83 ***	-0.86 ***
Shoot weight (g)[b]	0.21 *	-0.080 NS	-0.0080 NS	0.13 NS
Root weight (g)[b]	-0.035 NS	-0.048 NS	-0.12 NS	-0.015 NS

[a] Correlation coefficient. Spearman correlation used between nodule number and density. Pearson correlation used between all other variables.

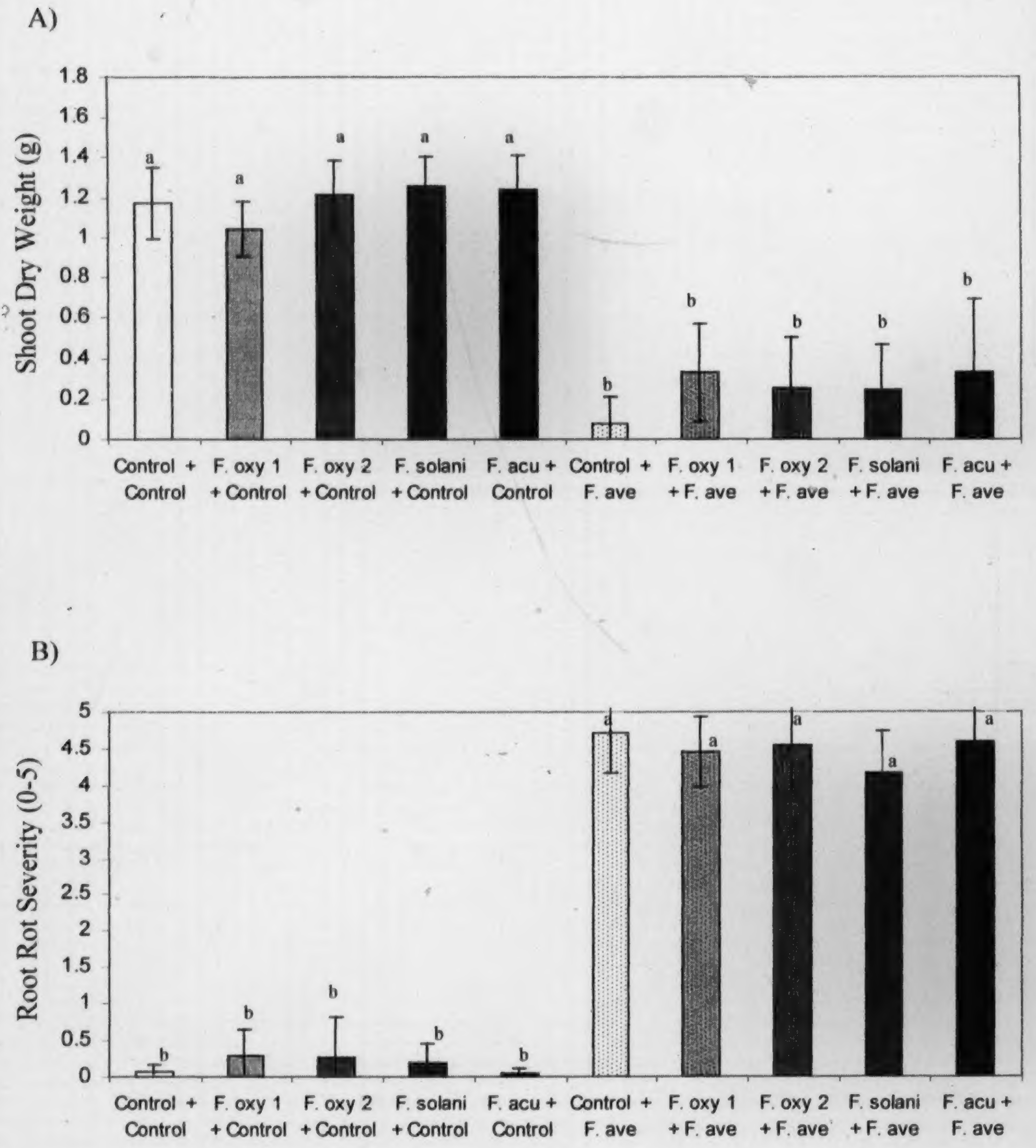
[b] Average per plant in the same pot.

NS Not significant; * Significant at $P \leq 0.05$; ** Significant at $P \leq 0.01$; *** Significant at $P \leq 0.001$

Table 4-3. Effect of artificial *F. avenaceum* seed contamination on lupins in 2006 at Lacombe, AB, Canada and in 2007 at Edmonton, AB, Canada

Treatment (% Seed Coated)	Seedling Establishment (average per row)	Yield (g)
0	99.7a	623.7a
5	97.3ab	578.4a
10	89.8ab	560.5ab
25	81.6bc	507.3ab
50	66.5cd	463.1abc
75	52.3d	400.7bc
100	32.6e	295.8c

Values within a column followed by the same letter do not differ based on Tukey-Kramer pairwise comparisons at $P \leq 0.05$


A)
Shoot Dry Weight (g)
1.8
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
a
a
a
a
a
b
b
b
b
b
Control + Control
F. oxy 1 + Control
F. oxy 2 + Control
F. solani + Control
F. acu + Control
Control + F. ave
F. oxy 1 + F. ave
F. oxy 2 + F. ave
F. solani + F. ave
F. acu + F. ave
B)
Root Rot Severity (0-5)
5
4.5
4
3.5
3
2.5
2
1.5
1
0.5
0
b
b
b
b
b
a
a
a
a
a
Control + Control
F. oxy 1 + Control
F. oxy 2 + Control
F. solani + Control
F. acu + Control
Control + F. ave
F. oxy 1 + F. ave
F. oxy 2 + F. ave
F. solani + F. ave
F. acu + F. ave

(continued)

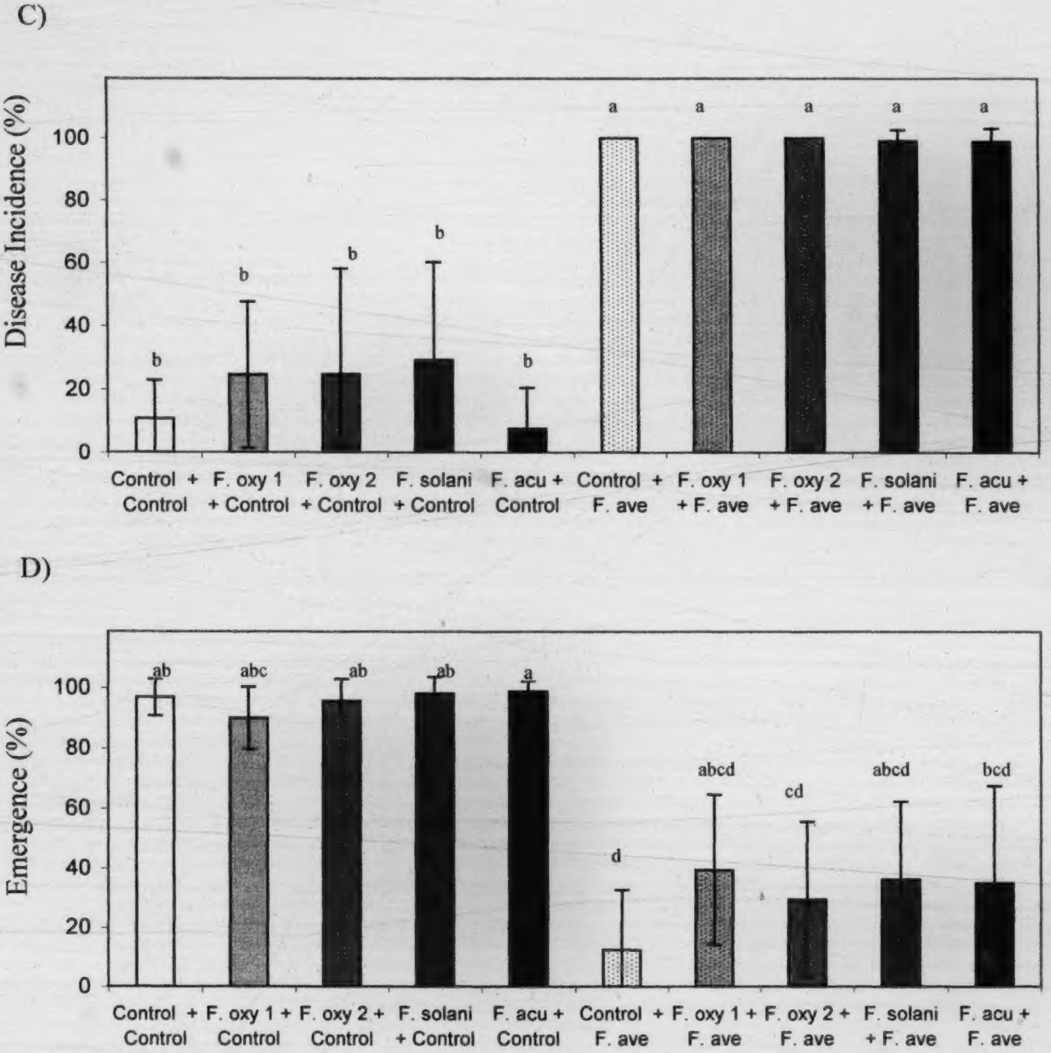
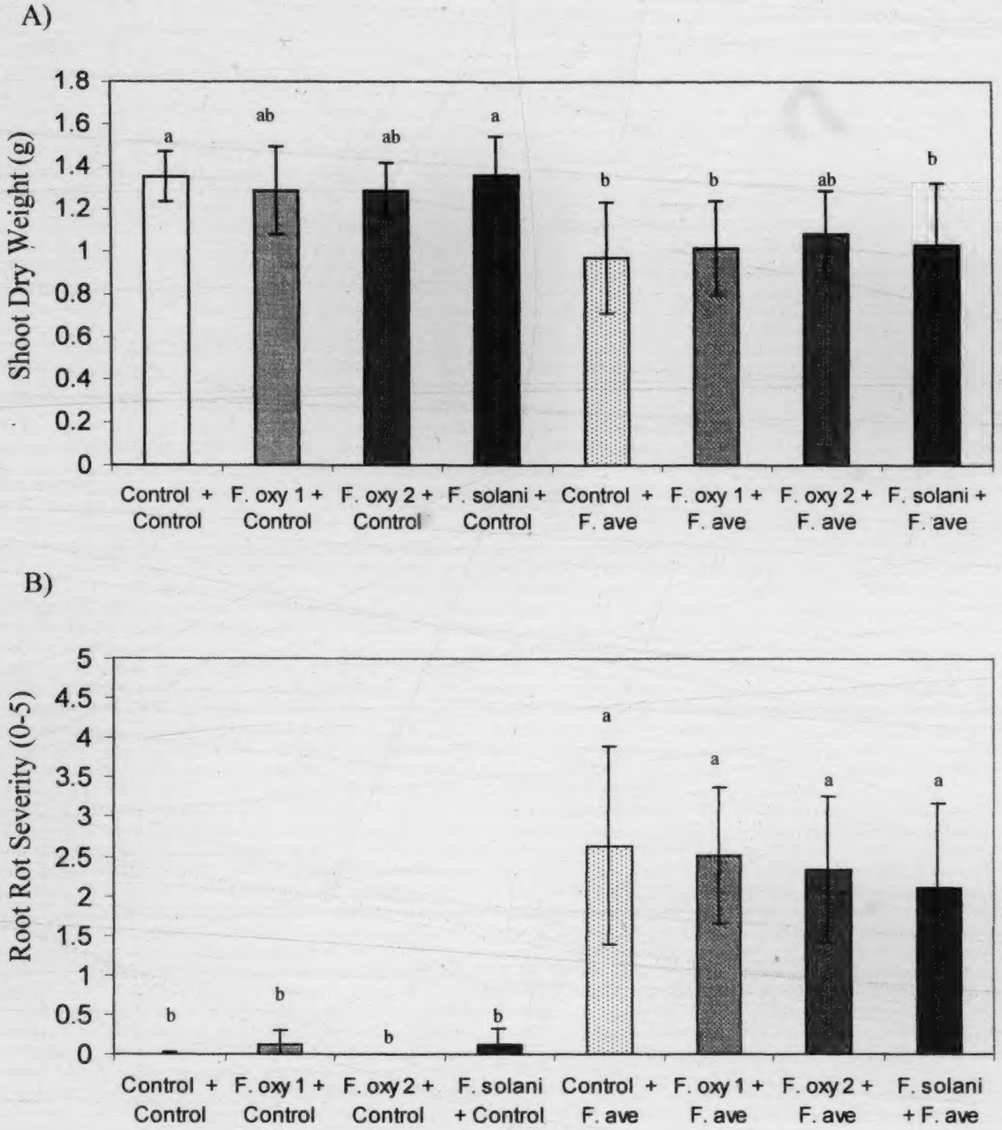


Figure 4-3: The effect of *Fusarium* isolates alone and in combination with *Fusarium avenaceum* (2×10^4 CFU g^{-1}) on *Lupinus angustifolius* cv. Arabella: (A) dry shoot weight (3 week old plants) (B) root rot severity; (C) disease incidence; and (D) seedling emergence at 2 weeks. Data represent the least significant (LS) means and standard deviations (bars) from two independent experiments. Bars in the same graph with the same letters are not significantly different according to Tukey-Kramer pairwise comparisons at the 95% confidence level. F. oxy 1 = *F. oxysporum* isolate 145, F. oxy 2 = *F. oxysporum* isolate 351, F. ave = *F. avenaceum*, F. acu = *F. acuminatum*


A)
Shoot Dry Weight (g)
1.8
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
a
ab
ab
a
b
b
ab
b
Control + Control
F. oxy 1 + Control
F. oxy 2 + Control
F. solani + Control
Control + F. ave
F. oxy 1 + F. ave
F. oxy 2 + F. ave
F. solani + F. ave
B)
Root Rot Severity (0-5)
5
4.5
4
3.5
3
2.5
2
1.5
1
0.5
0
b
b
b
b
a
a
a
a
Control + Control
F. oxy 1 + Control
F. oxy 2 + Control
F. solani + Control
Control + F. ave
F. oxy 1 + F. ave
F. oxy 2 + F. ave
F. solani + F. ave

(continued)

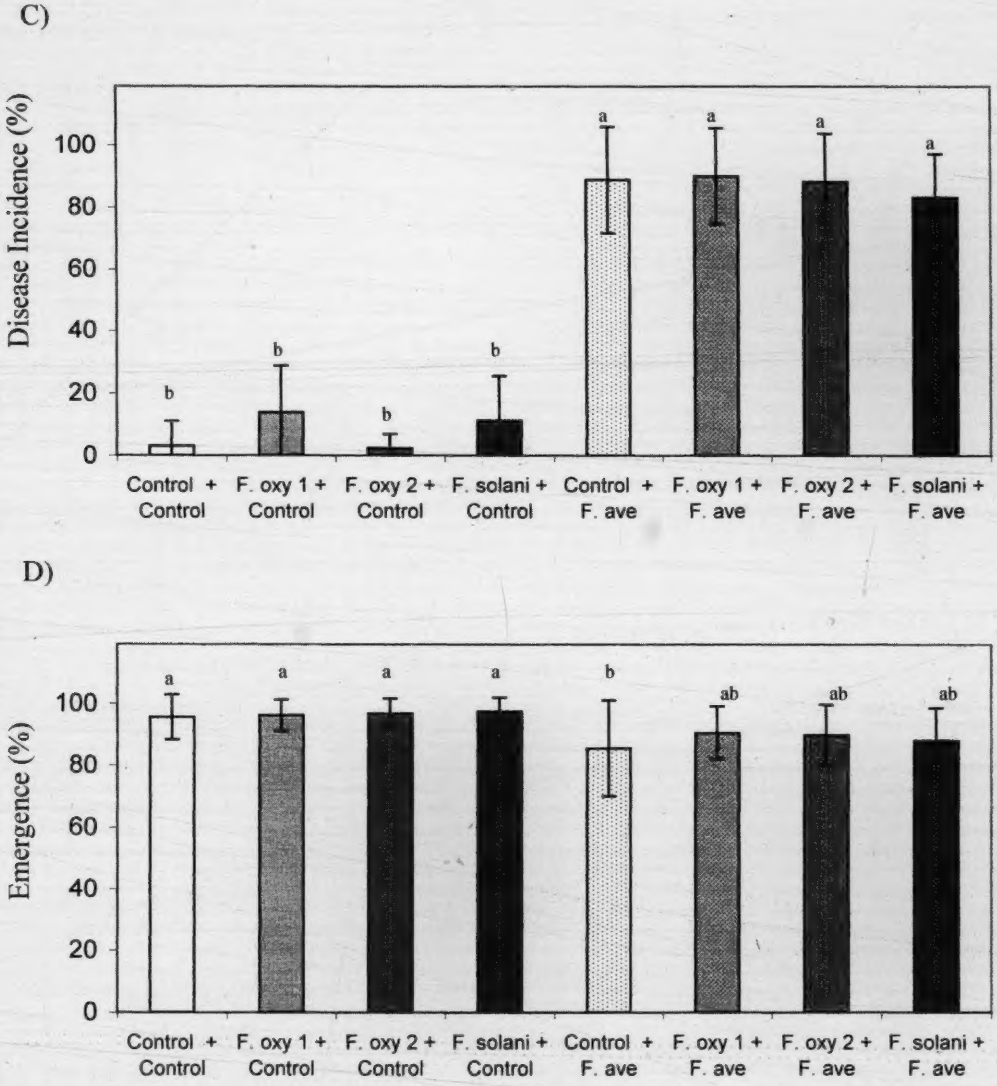


Figure 4-4: The effect of *Fusarium* isolates alone and in combination with *Fusarium avenaceum* (5×10^3 CFUs g^{-1}) on *Lupinus angustifolius* cv. Arabella: (A) dry shoot weight (3 week old plants) (B) root rot severity; (C) disease incidence; and (D) seedling emergence at 2 weeks. Data represent the least significant (LS) means and standard deviations (bars) from two independent experiments. Bars in the same graph with the same letters are not significantly different according to Tukey-Kramer pairwise comparisons at the 95% confidence level. F. oxy 1 = *F. oxysporum* isolate 145, F. oxy 2 = *F. oxysporum* isolate 351, F. ave = *F. avenaceum*.

Table 4-4. Inhibition of *F. avenaceum* by other *Fusarium* spp. isolated from lupins in dual culture on potato-dextrose agar (PDA) medium

	RGI[a]		Zi[b]	
	6 days	12 days	6 days	12 days
F. acuminatum	6.60±5.59 a	31.80±6.46 ab	10.75±2.38 a	2.125±1.27 a
F. oxysporum 1	7.75±4.40 a	30.21±7.11 ab	3.44±1.27 b	0±0 b
F. oxysporum 2	7.41±7.09 a	36.79±2.90 a	5.44±10.04 ab	0±0 b
F. solani	7.35±4.42 a	25.40±14.17 b	2.25±0.85 b	0.125±0.25 b
F. avenaceum	7.71±7.78 a	32.19±4.99 ab	2.63±2.38 b	0±0 b
PDA	1.56±4.04 a	-0.52±4.56 c	-	-

[a] RGI, inhibition of radial growth of F. avenaceum

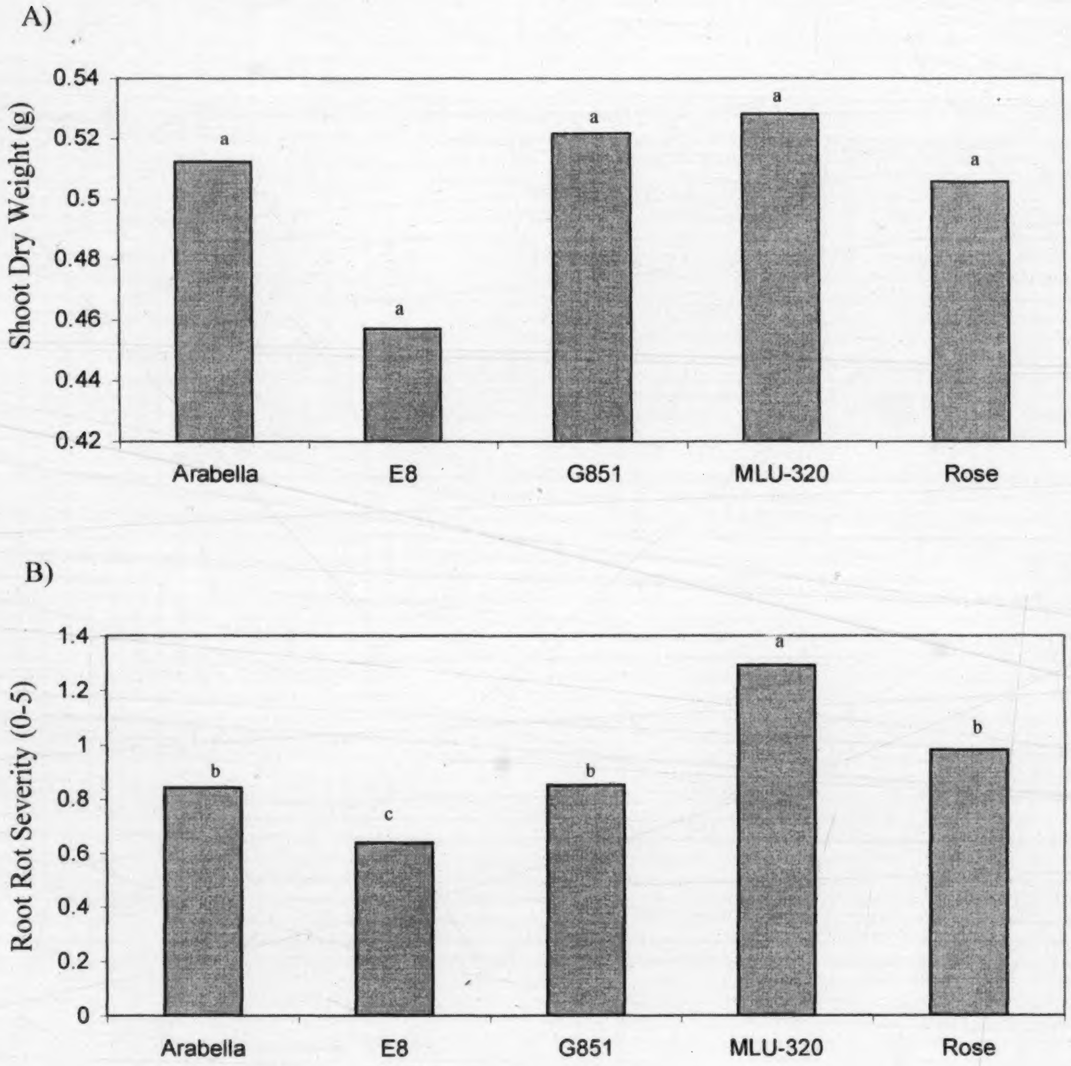
[b] ZI, the zone of inhibition between *F. avenaceum* and the other *Fusarium* isolate

Values are the mean of eight replications, ± standard deviation of the mean. Means followed by the same letter in each column are not significantly different according to Tukey-Kramer pairwise comparisons at the 95% confidence level.

Table 4-5. PROC MIXED test statistics for the effect of lupin genotype and *Fusarium* isolate on dry shoot weight, root rot severity, disease incidence, and emergence in the greenhouse.

Effect	DF_N	DF_D	F	*P* value
Shoot Weight				
Genotype	4	4.181484	4.519895	0.081793
Isolate	14	14.26339	12.93862	9.76E-06
Isolate*Genotype	56	55.93511	1.40848	0.101633
Root Rot				
Genotype	4	58.62838	29.85637	1.49E-13
Isolate	14	14.01775	18.91266	1.04E-06
Isolate*Genotype	56	58.54488	1.454591	0.079089
Disease Incidence				
Genotype	4	4.000902	13.53078	0.013548
Isolate	14	13.99999	36.65089	1.39E-08
Isolate*Genotype	56	1092	1.312717	0.064238
Emergence				
Genotype	4	1096	9.584614	1.28E-07
Isolate	14	14.00224	8.759795	0.000116
Isolate*Genotype	56	1096	1.047128	0.383083

DF_N = degrees of freedom for the numerator, and DF_D = degrees of freedom for the denominator.


A)
Shoot Dry Weight (g)
0.54
0.52
0.5
0.48
0.46
0.44
0.42
a
a
a
a
a
Arabella
E8
G851
MLU-320
Rose
B)
Root Rot Severity (0-5)
1.4
1.2
1
0.8
0.6
0.4
0.2
0
b
c
b
a
b
Arabella
E8
G851
MLU-320
Rose

(continued)

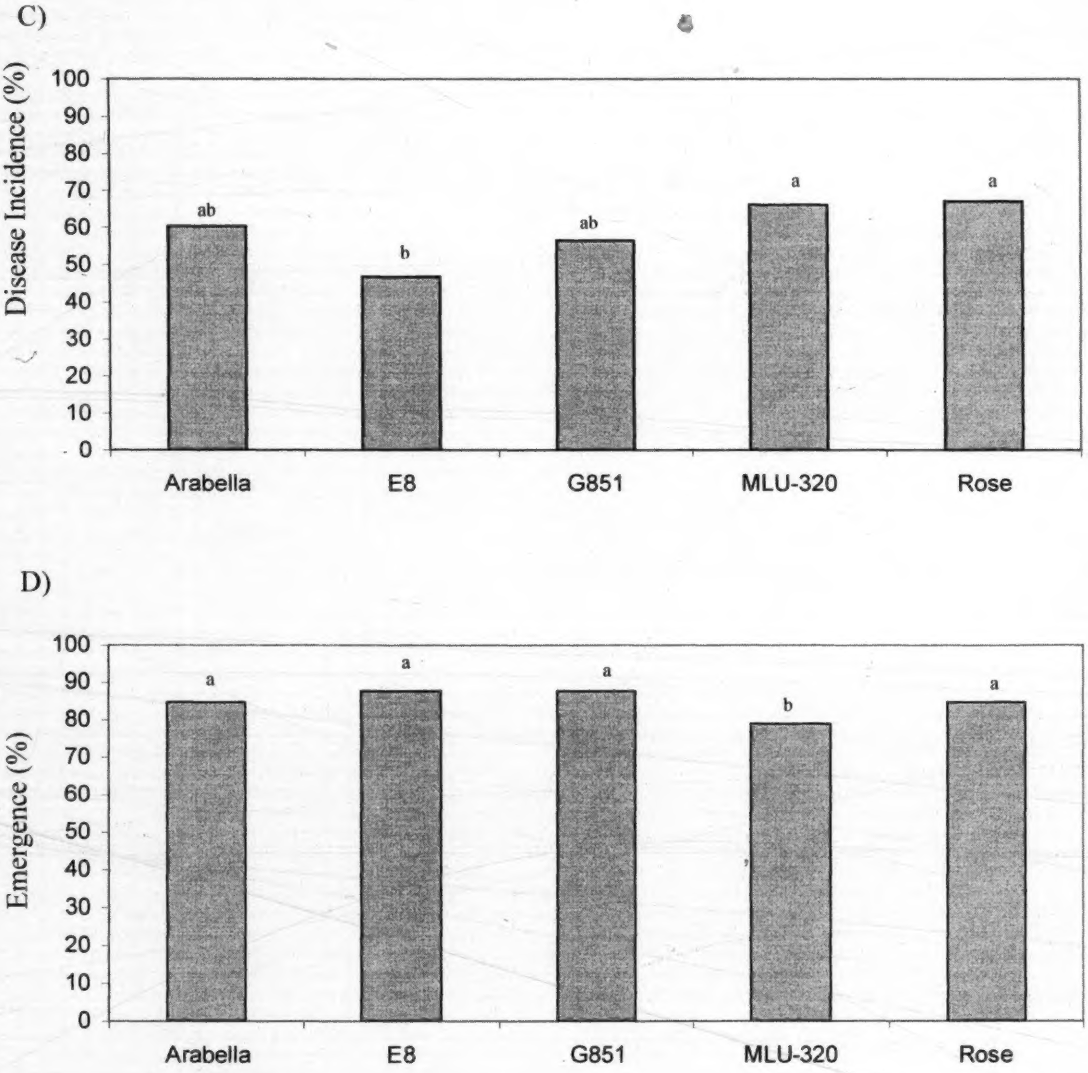
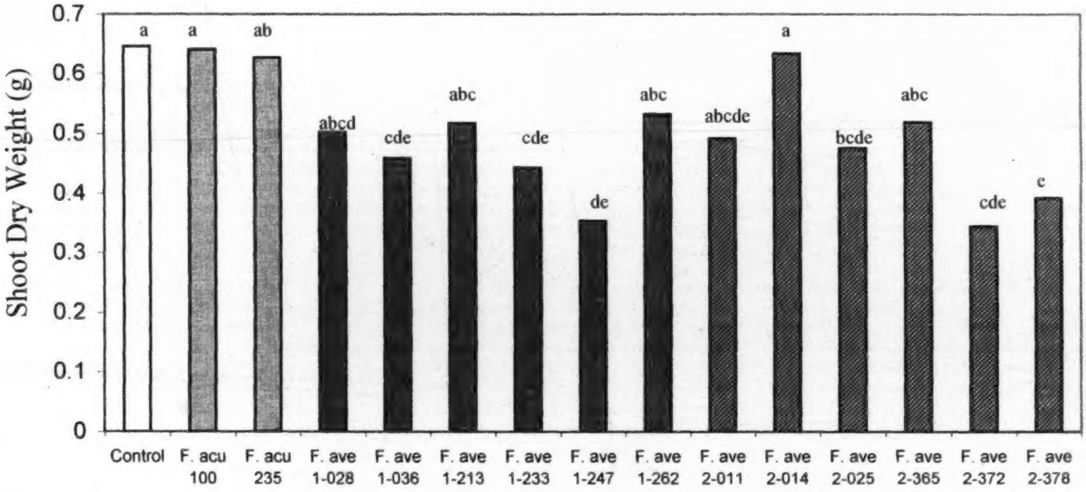


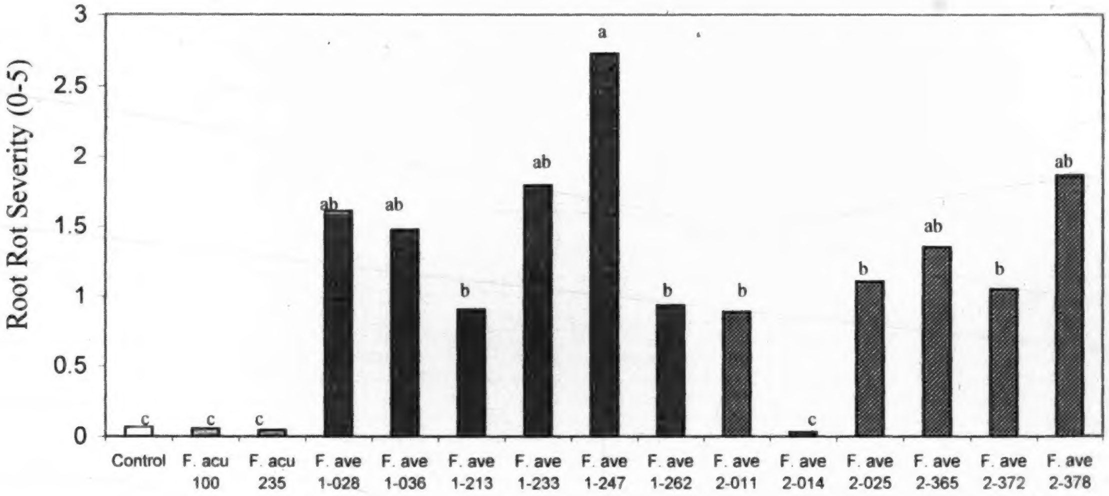
Figure 4-5. Reaction of five lupin genotypes to inoculation with *Fusarium*.

Data were combined from 14 *Fusarium* isolates to show their impact on (A) dry shoot weight (3 week old plants); (B) root rot severity; (C) disease incidence; and (D) seedling emergence (at 2 weeks). Data represent the least significant (LS) means from two independent experiments. Bars in the same graph with the same letters are not significantly different according to Tukey-Kramer pairwise comparisons at the 95% confidence level. Root rot data were transformed using $\log_{10}$ (x+1) and disease incidence were arcsine transformed. Back transformed values are shown.

A)



B)



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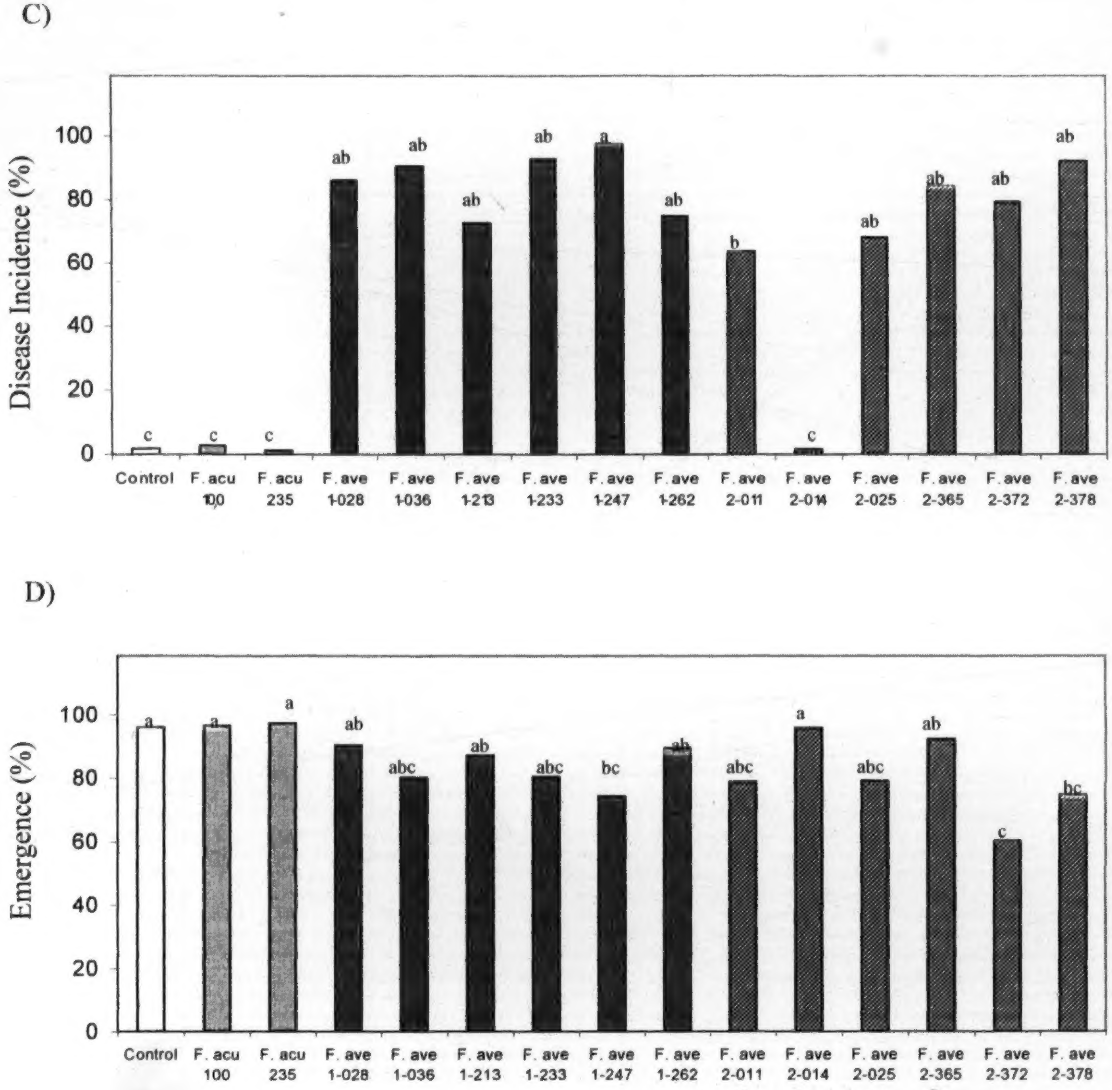

Figure 4-6. Reaction of lupins to twelve *F. avenaceum* isolates, two *Fusarium acuminatum* isolates, and a control.

Data were combined from 5 lupin genotypes to show the impact on (A) dry shoot weight (3 week old plants); (B) root rot severity; (C) disease incidence; and (D) seedling emergence (at 2 weeks). Data represent the least significant (LS) means from two independent experiments. Bars in the same graph with the same letters are not significantly different according to Tukey-Kramer pairwise comparisons at the 95%

confidence level. Root rot data were transformed using $\log_{10}(x+1)$ and disease incidence were arcsine transformed. Back transformed values are shown.

Table 4-6. Shoot weights, root rot, disease incidence, and emergence of five lupin genotypes inoculated with *F. avenaceum*.

	Shoot Weight (g)			Root Rot (0-5 scale)			Disease Incidence (%)			Emergence (%)		
Genotype	Group I	Group II		Group I	Group II		Group I	Group II		Group I	Group II	
Arabella	0.46	0.49	NS	1.76	0.99	***	81.0	60.2	***	82.5	80.8	NS
E8	0.44	0.43	NS	1.26	0.94	*	69.1	50.8	***	87.7	83.3	NS
G851	0.49	0.49	NS	1.64	1.22	**	76.9	60.0	***	87.1	83.5	NS
MLU	0.48	0.50	NS	2.52	1.88	***	83.3	64.8	***	76.0	75.4	NS
Rose	0.47	0.47	NS	1.94	1.35	***	86.3	67.9	***	85	77.5	*
Average	0.47	0.48	NS	1.83	1.28	***	79.3	60.8	***	83.7	80.1	NS

Note: Number of observations per mean, N=96. Number of observations for the average N=480.

NS = nonsignificant; * = significant at $P \leq 0.05$; ** = significant at $P \leq 0.01$; *** = significant at $P \leq 0.001$

Table 4-7. Effect of soil inoculation with *F. avenaceum* on emergence, root rot severity, root length, plant height, fresh weight, and dry weight of eighteen crop species.

	Emergence		Severity (0-9)	
Crop	Uninoculated	Inoculated	Uninoculated	Inoculated
Alfalfa cv. Anchor	7.8	0.6 *	0	8.6 *
Bean cv. CDC Pintium	8.4	8.8	0	0
Birdsfoot trefoil	8.8	1.2 *	0	8.5 *
Chickpea cv. Chico	7.0	0.3 *	0.2	8.9 *
Chickpea cv. Myles	8.4	3.8 *	0.11	0.96
Clover cv. Yellow Blossom	9.3	0.4 *	0	8.7 *
Fababean cv. Snowbird	4.1	1.1 *	2.8	8.7 *
Lentil – black	9.3	0.4 *	0	8.7 *
Lentil – green	9.2	0.4 *	0.07	8.9 *
Lentil – red	9.8	1.4 *	0.02	8.1 *
Lupin cv. Arabella	8.2	2.8 *	0.07	7.1 *
Pea cv. Cutlass	9.3	3.9 *	0.12	0
Soybean cv. Gaillard	7.8	5.6 *	0	0
Barley cv. Harrington	9.7	7.3 *	0	0
Barley cv. Vivar	9.3	6.0 *	0	0
Oats – hulled	9.7	9.4	0	0
Oats – hulless	7.6	3.2 *	0	0
Rye cv. AC Rifle	7.7	1.0 *	0	0
Triticale – spring	9.4	5.6 *	0	0
Triticale – winter	8.8	5.1 *	0	0
Wheat – spring	8.2	1.3 *	0	0
Wheat – winter	9.3	5.5 *	0	0
Canola – B. napus	7.6	2.6 *	0	6.9 *
Canola – B. rapa	8.9	0 *	4.5	9.0 *
Flax cv. Solin	7.3	0.4 *	0	8.6 *

(continued)

	Root Length (cm)		Plant Height (cm)	
Crop	Uninoculated	Inoculated	Uninoculated	Inoculated
Alfalfa cv. Anchor	13.0	0.5 *	7.7	0.2 *
Bean cv. CDC Pintium	12.6	12.3	16.1	15.3
Birdsfoot trefoil	12.9	0.3 *	6.0	0.4 *
Chickpea cv. Chico	12.5	0.3 *	15.0	0.2 *
Chickpea cv. Myles	16.0	6.0 *	15.5	3.1 *
Clover cv. Yellow Blossom	13.9	0.4 *	7.9	0.1 *
Fababean cv. Snowbird	8.1	1.3 *	7.3	0.6 *
Lentil – black	8.7	0 *	10.6	0.4 *
Lentil – green	16.6	0.5 *	15.7	0.4 *
Lentil – red	16.5	2.2 *	16.0	1.1 *
Lupin cv. Arabella	14.4	5.6 *	13.1	3.0 *
Pea cv. Cutlass	15.8	7.8 *	13.8	3.1 *
Soybean cv. Gaillard	12.0	9.3	7.0	4.0
Barley cv. Harrington	17.7	15.1	43.0	21.0 *
Barley cv. Vivar	17.8	14.3 *	40.0	20.6 *
Oats – hulled	19.4	17.3	35.6	32.0 *
Oats – hulless	15.5	5.9 *	22.0	8.5 *
Rye cv. AC Rifle	16.7	2.1 *	22.7	2.3 *
Triticale – spring	21.0	14.0 *	34.7	16.5 *
Triticale – winter	17.1	10.1 *	25.1	12.9 *
Wheat – spring	16.3	2.9 *	28.9	3.4 *
Wheat – winter	22.2	14.7 *	29.1	14.2 *
Canola – B. napus	7.0	3.1 *	5.0	1.1 *
Canola – B. rapa	4.1	0 *	8.6	0 *
Flax cv. Solin	8.4	0.4 *	12.3	0.4 *

(continued)

	Fresh Weight (g)		Dry Weight (g)	
Crop	Uninoculated	Inoculated	Uninoculated	Inoculated
Alfalfa cv. Anchor	0.95	0.020 *	1.06	0.017 *
Bean cv. CDC Pintium	9.49	7.95 *	7.67	7.67
Birdsfoot trefoil	0.41	0.009	0.46	0.011
Chickpea cv. Chico	3.84	0.03 *	4.54	0.06 *
Chickpea cv. Myles	4.44	0.98 *	5.17	1.0 *
Clover cv. Yellow Blossom	0.75	0.012	0.82	0.006 *
Fababean cv. Snowbird	5.84	0.31 *	4.54	0.30 *
Lentil – black	1.29	0.041 *	1.84	0.053 *
Lentil – green	3.66	0.053 *	4.14	0.048 *
Lentil – red	3.33	0.14 *	4.31	0.16 *
Lupin cv. Arabella	4.42	1.24 *	4.47	1.26 *
Pea cv. Cutlass	4.93	1.79 *	4.06	1.41 *
Soybean cv. Gaillard	1.85	1.17	10.2	1.17 *
Barley cv. Harrington	5.99	2.86 *	5.97	2.40 *
Barley cv. Vivar	6.36	3.41 *	6.08	3.13 *
Oats – hulled	3.78	2.58 *	2.63	2.20
Oats – hulless	2.00	0.70 *	1.83	0.52 *
Rye cv. AC Rifle	3.86	0.34 *	3.60	0.22 *
Triticale – spring	5.33	2.35 *	4.94	2.34 *
Triticale – winter	2.36	0.94 *	2.47	1.08 *
Wheat – spring	2.21	0.37 *	2.42	0.31 *
Wheat – winter	2.79	1.42 *	2.61	1.47 *
Canola – B. napus	2.30	1.33 *	1.94	1.17
Canola – B. rapa	2.17	0 *	2.35	1.52
Flax cv. Solin	0.73	0.025	0.65	0.014

Note: Number of observations per mean, N=10. Significant ($P \leq 0.05$) differences between inoculated and uninoculated treatments for the same cultivar are indicated by an asterisk.

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5. General discussion

Fusarium root rot is currently the most severe disease of lupins in Alberta (Chang et al. 2005, 2006). Isolations from diseased lupin roots in 2006 indicated that *Fusarium* was the main colonizer of lupin roots and *F. avenaceum* was the most abundant species. Pathogenicity screening showed *F. avenaceum* to be the only severe *Fusarium* pathogen of lupins (Chapter 2). The range in pathogenicity amongst *F. avenaceum* was large, with some isolates being non-pathogenic. The two lupin cultivars being commercialized in Alberta suffered comparable amounts of root rot. Both the isolation and screening for diseases may have been biased by the exclusive use of cultivars that were bred for *Fusarium* resistance. If susceptible cultivars had been available, it is possible that the results would have been different.

Genetic analysis showed *F. avenaceum* to be very diverse (Chapter 3), similar to the results of European researchers (Yli-Mattila et al. 1996; Turner et al. 1998; Chełkowski et al. 1999; Satyaparasad et al. 2000; Golińska et al. 2002). The cause of the division of *F. avenaceum* into two distinct groups, unrelated to time of DNA extraction, geographic origin, or host, is not clear. It is possible that the two groups are of phylogenetic significance and are related to the two "main groups" seen in Finnish *F. avenaceum* populations (Yli-Mattila et al. 1996). The presence of both mating types and linkage equilibrium suggests that sexual recombination occurs, or has occurred, in *F. avenaceum* in central Alberta (Chapter 3).

There were many aspects of the population analysis of *F. avenaceum* that could have been improved upon. The sample size was relatively small, limiting the number of analyses that could be performed. A larger sample size, greater than 100 isolates, would have been more informative. Index of association tests are typically not affected by small sample sizes, but other analyses could not be performed. Nonetheless, the number of polymorphic markers found amongst the *F. avenaceum* isolates was able to distinguish all isolates and were greater in number than are typically required to analyze fungal populations (McDonald 1997). Despite this, the inclusion of different types of markers, such as gene sequencing, would have allowed for a more robust and reliable analysis. The sampling of *F. avenaceum* isolates was also suboptimal for the population analysis that was attempted. Focusing solely on diseased lupin roots, although relevant to diseases of lupins, was not the most logical methodology for understanding central Albertan *F. avenaceum* populations. Focusing on only one host plant species for a species with an extremely large host range and the ability to survive saprophytically may have excluded certain genotypes, particularly if the population included members that colonize plants and cause disease less frequently. This prevents reliable interpretation of the results to the species as a whole. Due to the general lack of knowledge about the ecology of *F. avenaceum*, a hierarchal sampling design, not limited to the borders of lupin plots, would have been potentially more informative for deducing the population structure of the species. This, however, would not have been practical. Further research would be required to fully understand the structure and processes occurring within Albertan *F. avenaceum* populations.

More detailed analysis of the pathogenicity of *F. avenaceum* isolates (Chapter 4) revealed a strong relationship between the inoculum density of *F. avenaceum* and the disease severity and amount of nodulation in lupins. The ability of *F. avenaceum* to reduce the amount of nodulation of lupins may reduce the usefulness of the crop if the disease if not controlled. With the ability to symbiotically fix nitrogen considered one of the crops strongest assets, a disease that interferes with this may make the crop less desirable. Artificial seed inoculation with *F. avenaceum* was found to cause decreased emergence and yield. *Fusarium avenaceum* was capable of causing disease in lupins by itself and did not form a disease complex with other lupin species. Other *Fusarium* species decreased the severity of disease caused by *F. avenaceum*, but did not seem to be directly antagonistic to *F. avenaceum*. A more detailed study of *F. avenaceum* from the two UPGMA groups indicated that one group was capable of causing more severe root rot, whereas the other group was more capable of reducing emergence. This suggests that one *F. avenaceum* group may have been better adapted to causing disease early in seeds and young seedlings, and the other in older lupins. The results conflicted with the results of the earlier pathogenicity screenings (Chapter 2), which could have been to the different number of isolates used. Due to the extremely large host range of *F. avenaceum*, the division of *F. avenaceum* into multiple groups may be of importance to crops other than lupins. It is possible that certain subdivisions of Albertan *F. avenaceum* may behave differently when exposed to different crops or conditions. This could reduce the reliability of studies examining the use of different cultivars or fungicides against *F. avenaceum* if only a few or a single isolate of the species is included.

As a whole, the results from this study indicate that *F. avenaceum* is the dominant pathogen of lupin in central Alberta, and is a highly diverse one. The high level of genetic variation and the presence of both mating types suggest sexual recombination occurs within the pathogen populations. This may make genetic resistance to the disease difficult to achieve and maintain. The successful management of Fusarium root rot of lupin will probably benefit from an integrated management strategy, incorporating genetic resistance with proper disease control and agronomic practices.

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